KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI, GHANA

COLLEGE OF HEALTH SCIENCES
SCHOOL OF PUBLIC HEALTH
DEPARTMENT OF EPIDEMIOLOGY AND BIOSTATISTICS

ESTIMATION OF MALARIA TRANSMISSION INTENSITY IN SOUTHERN GHANA USING RAPID DIAGNOSTIC TEST DERIVED SERO-PREVALENCE RATES

BY

ALBERTA AMU QUARTEY (MBChB, MPH)

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A THESIS SUBMITTED TO THE DEPARTMENT EPIDEMIOLOGY AND BIOSTATISTICS, COLLEGE OF HEALTH SCIENCES, SCHOOL OF PUBLIC HEALTH, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY IN PUBLIC HEALTH

MAY 2016

DECLARATION

I hereby do declare that except for the references to other people's works, which have been duly acknowledged, this work is the result of my own original research.

I hereby also declare that this work had neither in whole nor in part been presented for the award of a degree elsewhere.

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SUPERVISOR

SIGNATURE……DATE…….

DR HARRY TAGBOR

HEAD OF DEPARTMENT

DEDICATION

To

Naa Kwarley; the light, Joshua; the thinker and Joel; the "mad" scientist!

ACKNOWLEDGEMENT

I wish to appreciate my Supervisors Professors Tsiri Agbenyega (KNUST) and David Schellenberg (LSHTM) for the leadership, support and supervision they provided for this work.

I am indebted to all participants who volunteered for this study, fieldworkers, laboratory staff, drivers and all other project staff, who collected and processed the data. To Dr. Evelyn Ansah; I can only say thank you.

I am also indebted to Mr. Victor Asoala, especially technical support for the Entomology work.

My gratitude also goes to Dr. Michael Ofori and his staff at the Immunology Department where I run the ELISAs and Dr. Samuel Dadzie and his Team (Entomology) all of the Noguchi Memorial Institute for Medical Research (NMIMR).

I wish to thank Isaac Agbavor my Research Assistant, Charity Sikanku, Victoria Ampah Johnston, Prosper Amewose, Philomina Ashiatey, Grace Brenya and Timothy Essilfie, the staff of MiPlace, all of the Dodowa Hospital.

Special thanks go to Dr. Margaret Gyapong and her team at the Dodowa Health Research Center.

I am very thankful to the Malaria Capacity Development Consortium, which funded the work. Helen for always being there, Dr. Amit Bhasin and all the staff at the MCDC Secretariat at the London School of Hygiene and Tropical Medicine.

DEFINITION OF TERMS

Asexual cycle. The life cycle of the malaria parasite in the host, from merozoite

Asymptomatic parasitaemia. The presence of asexual parasites in the blood without symptoms of illness.

Bias. A systematic difference from the truth, representing a source of error in estimating the association between exposure and outcome

Cohort Study. An observational study in which two groups are defined on the basis of their exposure to a potential risk factor and are followed up over time to measure the incidence of the outcome. This is then compared between the groups to give an estimate of relative risk

Cross sectional Study. An observational in which information on the outcome and exposure are measured at one point in time

Elimination. Reduction to zero of the incidence of infection by human malaria parasites in a defined geographical area, usually as a result of deliberate efforts.

Endemic. Applied to malaria when there is an ongoing, measurable incidence of cases and mosquito-borne transmission in an area over a succession of years.

Epidemic. Occurrence of cases in excess of the number expected in a given place and time.

Eradication. Permanent reduction to zero of the worldwide incidence of infection caused by human malaria parasites as a result of deliberate efforts. Intervention measures are no longer needed once eradication has been achieved.

Evaluation. Attempts to determine as systematically and objectively as possible the relevance, effectiveness and impact of activities in relation to their objectives.

Exposure. The act of being exposed to a potential risk or protective factor

Gametocyte. The sexual reproductive stage of the malaria parasite

Hypnozoites. The dormant stage of the malaria parasite present in the host's liver cells (limited to infections with *Plasmodium vivax* and *P. ovale).*

Hypothesis. A supposition phrased in such a way as to allow it to be tested and confirmed or refuted

Incidence. The number of new cases of an outcome that develop in a defined population of individuals at risk during a specified period of time.

Incubation period. The time between infection (by inoculation or

Intervention (public health). Activity undertaken to prevent or reduce the occurrence of a health condition in a population. Examples of interventions for malaria control include the distribution of insecticide-treated mosquito nets, indoor residual spraying with insecticides, and the provision of effective antimalarial therapy for prevention or curative treatment of clinical malaria.

invasion of red blood cells to schizont rupture. The duration is approximately 24 hours in *Plasmodium knowlesi,* 48 hours in *P. falciparum, P. malariae, P. ovale* and *P. vivax* and 72 hours in *P. malariae*.

Malaria case definition. A History of fever in the past 48 hours in which malaria parasites have been demonstrated in a patient's blood by microscopy or a rapid diagnostic test.

Malaria incidence. The number of newly diagnosed malaria cases during a specified time in a specified population.

Malaria-free. An area in which there is no continuing local mosquito borne malaria transmission and the risk for acquiring malaria is limited to introduced cases only.

Monitoring (of programs). Periodic review of the implementation of an activity, seeking to ensure that inputs, deliveries, work schedules, targeted outputs and other required actions are proceeding according to plan. otherwise) and the first appearance of clinical signs.

Passive Case Detection. Detection of malaria cases among patients who, on their own initiative, go to a health facility for treatment, usually for febrile disease.

Person time at risk. The sum of the time each individual in a defined population is at risk of an outcome. It is used as the denominator in the calculation of incidence rates

Population At Risk. Population living in a geographical area in which locally acquired malaria cases occurred in the current year and/or previous years.

present in the host's red blood cells.

Prevalence. The number of existing cases of an outcome in a defined population at a particular point in time divided by the total number of people in that population at the same time.

Rapid Diagnostic Test. An antigen-based stick, cassette or card test for malaria in which a coloured line indicates that plasmodial antigens have been detected.

Ratio. The relationship between two numbers of the same type expressed as a/b or a:b e.g. Prevalence ratio, rate ratio

Relative Risk. An estimate of the magnitude of association between exposure and incidence of an outcome, and can be interpreted as the likelihood of developing an outcome in those exposed compared to those unexposed.

Ring stage. Young, usually ring-shaped, intra-erythrocytic malaria parasites, before malaria pigment is evident by microscopy.

Schizont. Mature malaria parasite in host liver cells (hepatic schizont) or red blood cells (erythrocytic schizont) that is undergoing nuclear division by a process called schizogony.

Sensitivity. The proportion of individuals who truly have an outcome that are correctly identified by a screening or diagnostic method

Severe anaemia. Haemoglobin concentration of < 5g/100 mL (haematocrit < 15%).

Severe *falciparum* malaria. Acute falciparum malaria disease with signs of severity and or evidence of vital organ dysfunction.

Specificity. The proportion of individuals who truly do not have an outcome that are correctly identified by a screening or diagnostic method

Sporozoite. Motile malaria parasite that is infective to humans, inoculated by a feeding female anopheline mosquito that invades hepatocytes.

Transmission intensity. This is the frequency with which people living in an area are bitten by anopheline mosquitoes carrying human malaria sporozoites. It is often expressed as the annual entomological inoculation rate, which is the average number of inoculations with malaria parasites received by one person in 1 year.

Trophozoite. The stage of development of malaria parasites growing within host red blood cells from the ring stage to just before nuclear division. Mature trophozoites contain visible malaria pigment.

Uncomplicated malaria. History of Fever (or observed) or other symptomatic malaria parasitaemia with no signs of severity and or evidence of vital organ dysfunction.

Vectorial capacity. Number of potential new infections that the population of a given anopheline mosquito vector would distribute per malaria case per day at a given place and time.

LIST OF ABBREVIATIONS/ACRONYMS

ACTs	*Artemisinin based Combination Therapies*
ADAMSEL	*Auditable Data Analysis and Management System for ELISA*
AS02	*Adjuvant System 02*
CFT	*Complement Fixation Test*
CHPS	*Community-based Health Planning and Services*
CHRPE	*Committee on Human Research, Publications and Ethics*
CI	*Confidence interval*
CSP	*Circumsporozoite Protein*
DDT	*Dichloro-Diphenyl-Trichloroethane*
DSS	*Demographic Surveillance System*
EIR	*Entomological Inoculation Rate*
ELISA	*Enzyme Linked Immunoabsorbent Assay*
G6PD	*Glucose-6-Phosphate Dehydrogenase*
GHS	*Ghana Health Service*
Global Fund	*Global Fund to Fight AIDS, Tuberculosis and*
HBI	*Human Blood Index*
HDSS	*Health and Demographic Surveillance System*
HLCs	*Human Landing Catches or Collections*
HRP2	*Histidine-rich protein 2*
IFAT	*Immunofluorescence Antibody Test*
IHA	*Indirect Heamaglutination Assay*
IgG	*Immunoglobulin G*
IM	*Intramuscular*
INESS	*INDEPTH Effectiveness and Safety Studies of Antimalarial in Africa*
IPTi	*Intermittent preventive treatment in infancy*

IPTp	*Intermittent Presumptive Treatment for malaria in pregnancy*
IRS	*Indoor Residual Spraying*
ITN	*Insecticide Treated Bed net*
IV	*Intravascular*
KNUST	*Kwame Nkrumah University of Science and Technology*
LLINs	*long-lasting insecticidal nets*
MBR	*Man Biting Rate*
MDGs	*Millennium Development Goal*
MICS	*Multiple Indicator Cluster Survey*
MIS	*Malaria Indicator Surveys*
MPAC	*Malaria Policy Advisory Committee*
MSP-1_{19}	*Merozoite Specific Protein 1_{19}*
NAA	*Nucleic Acid Assays*
NMCP	*National Malaria Control Program Ghana*
NMIMR	*Noguchi Memorial Institute for Medical Research*
OD	*Optical Densities*
OPD	*Outpatient Department*
P.	*Plasmodium*
PBS	*Phosphate Buffered Saline*
PCR	*Polymerase Chain Reaction*
PfHRP2	*Plasmodium falciparum histidine-rich protein-2*
pLDH	*parasite-lactate dehydrogenase*
ppy	*per person year*
PR	*Parasite Rate*
PSC	*Pyrethrum Spray Catches*
QA	*Quality Assurance*

RBC	*Red Blood Cells*
RBM	*Roll Back Malaria*
RDTs	*Rapid Diagnostic Tests for malaria*
RR	*Relative risk, or risk ratio*
SMC	*Seasonal malaria chemoprevention*
SP	*Sulphadoxine–Pyremethamine*
SPR	*Slide positivity rate*
T3	*Test, Treat, Track*
UNICEF	*United Nations Children's Fund*
WHO	*World Health Organization*

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ABSTRACT

An understanding of the epidemiology of malaria in an area is critical for the design and evaluation of control efforts. The Dangme West district has three distinct ecological zones, a forest (Dodowa sub district), a coastal zone (Prampram sub district) and a lakeside zone (Osudoku sub district). The current data for the Dangme West District of Ghana dates back to 1993, when the parasite prevalence ranged from 42% (dry season) to 51% (rainy season), while in Prampram the prevalence were 20% and 37% respectively. The annual Entomological Inoculation Rate (EIR) was 22 and 3.6 infective bites per person per year in Dodowa and Prampram respectively in 1993. Though many malaria interventions have been rolled out through research and program activities, there were no current data to ascertain the veracity of interventions and guide further program actions. The objective of this study was therefore to update and determine the complete the epidemiology of malaria in Dangme West district. The study was in 3 parts; ***Prevalence*****:** 2 cross-sectional surveys (6854 persons of both genders and all ages, selected using multistage cluster sampling) were conducted at the end of the wet (August 2011) and dry (March 2012) seasons. Participants were interviewed using questionnaires after which finger prick blood samples were taken for Rapid Diagnostic Tests (RDTs) and blood films to check for malaria. ***Incidence*****:** A cohort of 3156 participants of all ages, from the 3 zones was selected using multistage cluster sampling. Participants were followed once a month for a year. A history of fever within the past 2 weeks was elicited at each visit. Those who responded positively had finger prick blood samples taken for RDT and blood films. ***Entomology:*** 4 houses per zone were randomly selected for mosquito collection per month for a year using Human Landing catches. Ethical approval was obtained from Kwame Nkrumah University of Science and Technology Committee on Human Research, Publications and Ethics: CHRPE 189/10 and Ghana Health Service: GHSER:

03/5/11. Data were double entered in EPIDATA, cleaned and analyzed in STATA 12. There was minimal seasonality in blood slide positivity in the dry and wet seasons, with an average parasite prevalence of 6.5%, (Lakeside 2.7% to 8.5% Coastal). The dominant parasite specie was *Plasmodium falciparum* (96%). The agespecific parasite prevalence was 9% in 0-9 year olds, 8% in 10-19 year olds, 5% in 20-29 year olds, 3% in 30-39 years and 4% in those aged 40 years and above. The corresponding age-specific MSP-1_{19} prevalence was 37%, 58%, and 60%, 66% and 67% respectively. 8% of participants in the incidence study reported a history of fever in the last 48 hours, 3% used antimalarial for perceived fever and 6% had used an ITN the night before home visits. The Forest zone had an incidence rate of 85/1000py (slide), Coastal 41/1000py and Lakeside 13/1000py. The absence of a ceiling in a room was associated with an excess risk of 15%. The incidence rate was 119/1000py in 0-4 year olds, 136/1000py in 5-9 year, 50/1000py in 10-19 year, 9/1000py in 20-29 year, 18/1000/py in 30- 39 year olds and 24/1000py in those over 40 years. The district EIR was 81 infective bites per person year (ppy). April had the highest EIR of 1/pp/night. The Lakeside zone had an EIR of 100/ppy; Forest 81 ppy and Coastal 30ppy for the same period. The main vector species was *An. gambiae* s.l, which constituted 95%, with *An. funestus* Giles forming the rest. Overall rates had decreased by about 40% from the 1993 levels. The Lakeside zone had the lowest malaria incidence despite vast irrigated fields. The Forest zone, with the lowest verified ITN use, and the 5-9 year age group bore the brunt of morbidity. There is reduction in malaria burden in the area in the last 20 years, with more marked reduction in the Lakeside than at the Coastal and Forest. The Lakeside with the highest ITN use had the lowest parasite prevalence and incidence rates. ITN distribution and use need to be improved in the Forest and Coastal zones and access to testing and treatment with quality assured ACTs improved in the Osudoku zone. Research into effective combination of interventions to target the 5-9 and 10-19 year

groups need to be done to address disease burden and asymptomatic carriage in those age groups.

CHAPTER 1: INTRODUCTION

1.1: Background Information

Malaria is a preventable vector borne disease, globally estimated to have caused 198 million disease cases and 584,000 (367,000-755,000) deaths in 2013, most of these deaths (78%) occurred in children aged less than 5 years in Sub-Saharan Africa, where a child dies of malaria every minute. Ninety percent of all estimated malaria deaths occurred in Sub-Saharan Africa, even though Malaria mortality had decreased by at least 25% worldwide, and by over 33% in Sub-Saharan Africa in the last decade. Worldwide, about 3.2 billion people are at risk of developing the disease with about half of them being at high risk. (World Health Organization 2014; White *et al*. 2014, Feachem, 2009;). It is also the fifth top cause of deaths due to infectious diseases worldwide. Most of the disease burden occurs in Africa, where 80% of the global malaria cases occur in 17 countries and 80% of deaths occur in 14 countries. (Feachem, R.G.A 2009).

The last decade (2000-2010) saw renewed and increased effort at controlling malaria globally, though early efforts had already begun in the 1950s (World Health Organization 2012a; Steketee & Campbell 2010; O'Meara *et al*. 2010; Gething *et al*. 2014). Within this period, the 2011 World Health Organization (WHO) Malaria Report states that significant and durable achievements were made in reducing the malaria map, (though targets for 2010 were not achieved in the Sub- Saharan African region) saving about 1.1 million lives worldwide. Most of these prevented cases (52%) and lives saved (58%) happened in 10 countries, which had the highest estimated malaria burdens in 2000. Fifty percent (50%) of countries on the malaria map (reporting only 3% of cases) are on track to decrease incidence rates by 75% by 2015.

Control programs in the last decade have had their greatest impact where the burden is greatest, though the progress achieved seems to have plateau between 2010 and 2012 (World Health Organization 2012c). In the Sub-Saharan African region, weak surveillance and monitoring systems made it difficult for progress to be demonstrated. In a few countries, which included Zambia and Sao Tome, expansion of the use of microscopy and Rapid Diagnostic Tests (RDTs) for malaria has seen the numbers of confirmed cases to increase. Though the causes for this increase are undefined, they are a reflection of local diagnostic practice, strength of local malaria surveillance systems and the real underlying trends in malaria incidence (World Health Organization 2011; O'Meara *et al*. 2010; World Health Organization 2010c).

The percentage of households owning at least one insecticide treated bed net (ITN) in Sub-Saharan Africa rose from 3% in 2000 to 53% in 2011 and has remained the same for 2012. Ninety percent of these persons who own the ITNs use them (World Health Organization 2012c). Indoor Residual Spraying levels have also remained at 2011 levels in 2012. Globally, diagnostic testing for malaria has increased from 68% in 2005 to 77% in 2011 (increase between 2010 and 2011 only 1%) and has remained same, with the largest increases in Sub- Saharan Africa (World Health Organization 2012c). The year 2012 also saw the launch of the WHO Test, Treat and Track (T3) program to encourage endemic countries to scale up malaria diagnostic testing, treatment and surveillance.

In Sub-Saharan African countries, the lack of detailed investigations of trends in malaria transmission, cases and diagnostic practices makes it difficult to portray an accurate picture of the real changes in malaria burden. The situation is even worse in West Africa, where data on malaria trends are very scarce (Satoguina *et al*. 2009).

Increased control efforts demand accurate measurements of changes in epidemiology to justify control efforts as well as to improve interventions (Gething *et al*. 2014; Kusi *et al*. 2014). Changes in malaria epidemiology in countries with weak surveillance systems are difficult to detect and this affects the ability to respond appropriately with the right interventions. This is true especially in the more populous countries of Central and West Africa (World Health Organization 2011). Most studies on the trends in malaria epidemiology have concentrated on children 0-5 years. However, as malaria control measures intensifies and transmission decreases, malaria epidemiology in children 0-5 years may not reflect the true epidemiology at the community level and may not be useful especially for targeting of interventions.

Ghana is a high transmission country in Sub-Saharan Africa (GHS Annual Report, 2011). The major Plasmodium specie is *P. falciparum*, which causes 90% of morbidity. The major vectors are *An. gambiae, arabiensis* and *funestus* (Owusu-Agyei *et al*. 2009; Appawu *et al*. 2001; Appawu *et al*. 2004). In Ghana, the national intervention package includes Insecticide Treated bed nets (ITNs), Intermittent Presumptive Treatment for malaria in pregnancy (IPTp), case management using Artemisinin based Combination Therapies (ACT) and indoor residual spraying (IRS)(World Health Organization 2011; Ghana Health Service 2011a). In 2010, the ITNs distributed free of charge of covered 25-50% of the population at risk. For the first time, the Multiple Indicator Cluster Survey (MICS) in 2011, which covered children aged 0-5 years, from all ten regions of the country included malaria parasite prevalence (Ghana Statistical Service 2012).

1.2: Problem Statement

In the Dangme West District, a Ghana Health Service (GHS) designated health research site where malaria studies and control interventions have been carried out since the 1990s, there was very little current information on the levels of malaria transmission intensity and factors that modulate it at the community level.

1.3: Rationale of Study

Malaria transmission intensity data was lacking for the whole district. Where some data was available it was outdated. The most recent malaria epidemiological data on malaria in the district was over ten years old, though the last decade has seen intensified control efforts in Ghana and in the study area. In spite of these efforts, no comprehensive assessment of the current situation has been done. However, reported malaria from the outpatients departments at both public and private health facilities in the district remained high. The obvious question from the then District Director and team remained; are the high outpatient reports of malaria a true reflection of malaria burden in the district (Ghana Health Service 2011)?

There was therefore a critical need to characterize and estimate malaria transmission intensity to form the basis for further research and improve on program intervention targeting.

1.3.1 Conceptual Framework for Malaria Transmission Determinants

Malaria transmission in an area depends on the amount and duration of transmission (estimated by EIR) and the diversity of *Plasmodium* parasites in the locality. Climatic, malaria control and ecological factors affect the ability of the *Anopheline* vectors and *Plasmodium* parasites to cohabit adequately for transmission to occur. The density of *Anopheline* vector population, species types and infectivity as well as availability of sources of infection (gametocyte carriers, either humans or mammalian) determine the frequency of transmission. Host and parasite factors then determine parasite exposure (parasitaemia) and other clinical disease outcomes through a complex relationshiP. These relationships are explored through frameworks in Figures 1.1 and1.2.

Figure 1.1 Conceptual Framework on the determinants of Malaria Transmission

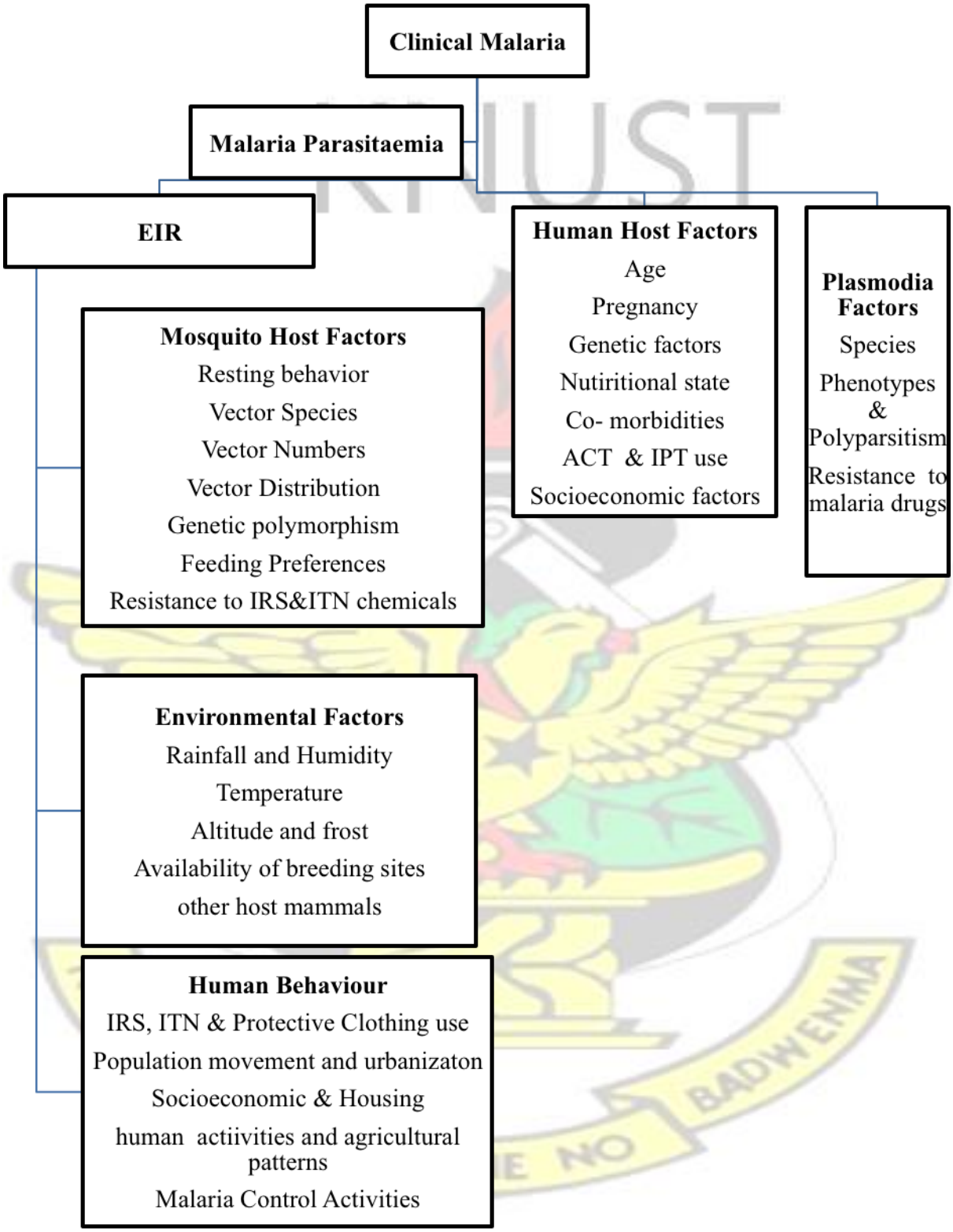
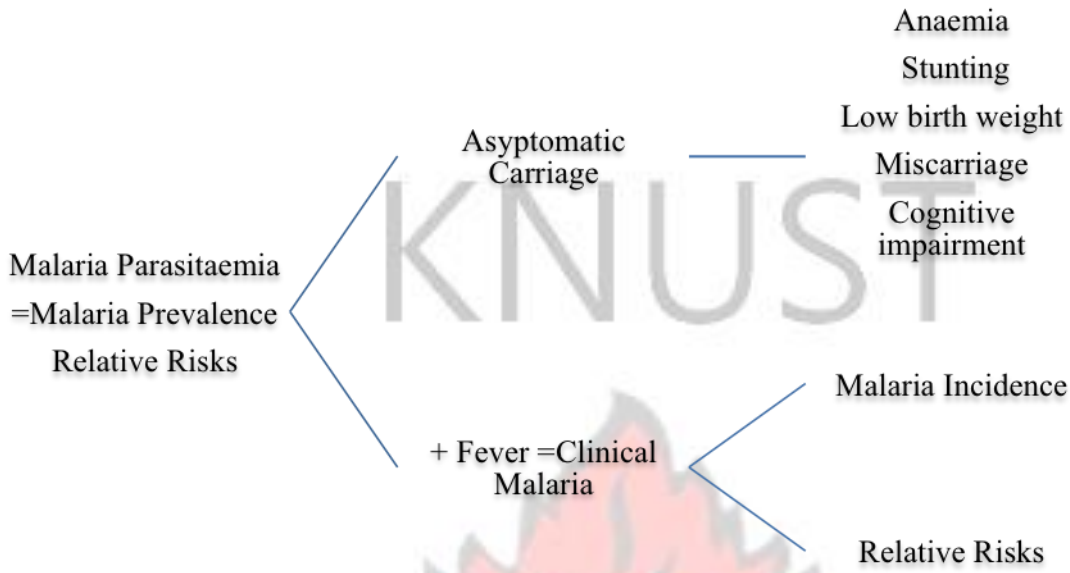


Figure 1.2 Conceptual framework on the impact and measurement of Malaria infection



1.4 Hypothesis

My hypotheses were:

- The use of malarial interventions has not affected the level of malaria transmission intensity at the community level.
- Malaria transmission intensity does not differ by ecological zone within the district**.**

1.5 Research Questions

This led to the following research questions:

- Has the coverage of malaria interventions at the community affected the malaria transmission burden?
- What is the utility of RDTs at the community level for field epidemiological testing of malaria?
- To what extent can serology determine the force of transmission of malaria at the community level?

1.6 General Objective

To describe the transmission intensity of Malaria in the Dangme West District of Ghana

1.7 Specific objectives

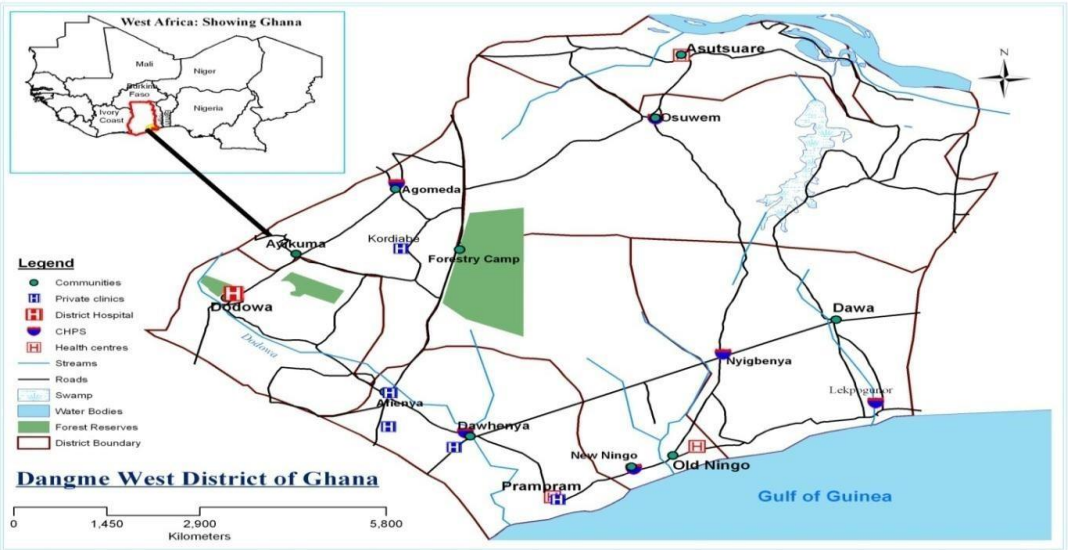
1. To describe the prevalence of malaria between August 2011 and April 2012, using repeated surveys, in the Dangme West District.
2. To determine the incidence of malaria in the district in the same period.
3. To estimate the transmission intensity using age specific malaria seroprevalence rates in the district derived from immuno-phoretic rapid diagnostic tests in the same period.
4. To estimate entomological inoculation rate (EIR) in the district within the same period.

1.8 Profile of Study Area

1.8.1 Study Area and Population

The study was conducted in the Dangme West District of Ghana. The district is zoned into 4 sub-districts and 7 area councils. Each house and household in the district is numbered with each member of a household bearing a unique identity number. The Dangme West District Health and Demographic Surveillance System (HDSS) follow the members of the households twice a year for vital events, migration and other demographic data. The HDSS started in 2005; two rounds of data collection are done each year(Gyapong *et al*. 2013).

Map 1: Map of the Dangme West District



Map of the Dangme West district showing geography and distribution of health facilities. The Prampram Zone is along the Coast, Dodowa is along the north Western corridor with forest cover and Osudoku is on the northeastern corridor with the least penetration of health facilities**.** The district has one hospital, four health centers, one mission clinic, seven community-based health planning and services (CHPS) compounds and seven private clinics.

1.8.2 Geography

The Dangme West district is one of the ten districts in the Greater Accra Region and one of the two purely rural districts in the region, with the largest land surface area (about 1,700 square kilometers). It is bounded on the north by the Akuapim ridges; on the south by the Gulf of Guinea; on the east by River Volta, South Tongu and the Dangme East District; and on West by the Tema Metropolitan Area.

The land is flat and at sea level with isolated hills. Among the hills are the ancient "Shai Hills" which are a tourist attraction. The vegetation is mainly coastal savannah with a dense thicket with forest type trees in the Dodowa sub-district (Dodowa Forest).

The district is divided into 4 administrative sub-districts,

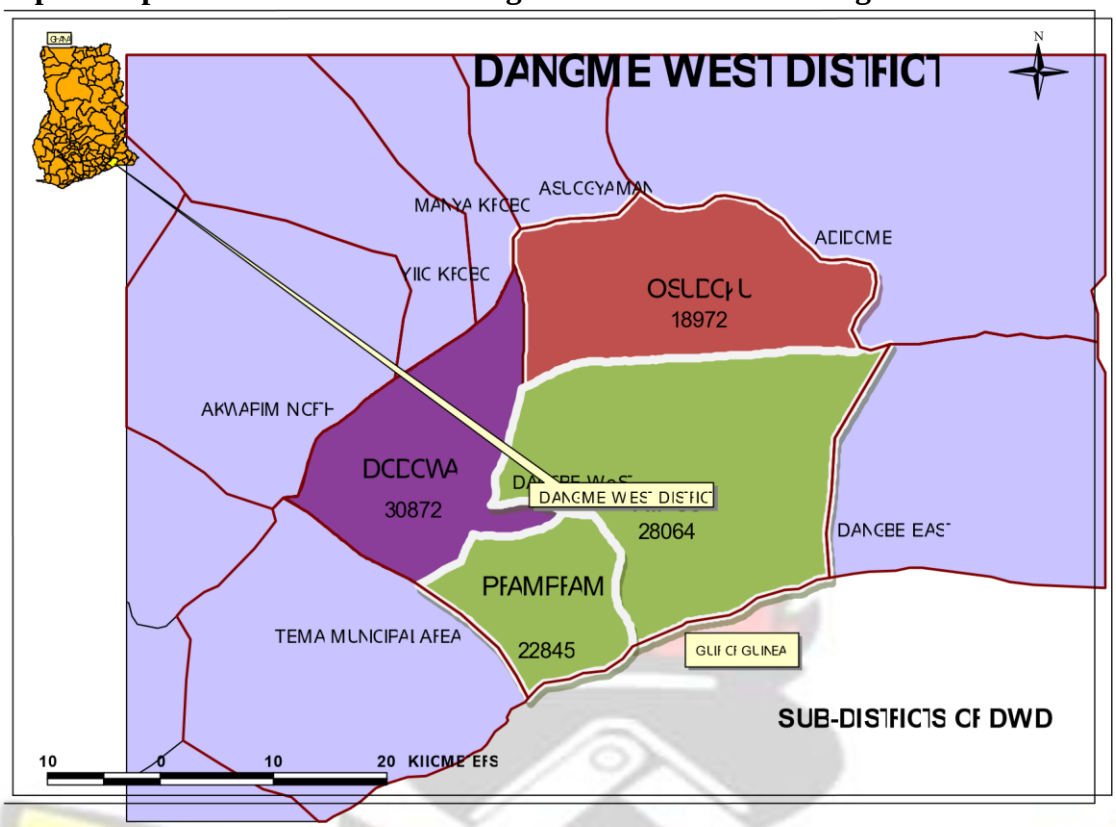
- Dodowa Sub-district
- Prampram Sub-district
- Ningo Sub-district
- Osudoku Sub-district

The administrative sub-districts corresponds to a large extent, to the 4 traditional areas of the district i.e. the Shai, Prampram, Ningo and Osudoku traditional areas. There are seven area councils, two in the Dodowa Sub-district (Dodowa and Ayikuma) one in Prampram, two at Ningo (Ningo and Dawa) and two at Osudoku (Asutuare and Osuwem) (Ghana Health Service 2011b).

Table 1.1: Ecological zones in the Dangme West District

Dodowa	Forest
Osudoku (Asutuare)	Lake side
Ningo	Coastal
Prampram	Coastal

Map 2: Map of the District of the Dangme West District showing sub-districts



Ghana Health Service Dangme West District 2011: The Map is as demarcated by the District Health Management Team by land area and population for 2011. Dodowa had a population of 30872, Osudoku 18972 and Prampram 22845. The population figures are projected from the 2010 Population and Housing Census.

The main road networks passing through the district such as the Dodowa-Somanya road, Accra-Aflao road and Accra–Akosombo road are in very good condition. In the rainy season most of the villages are inaccessible except with a four-wheel drive.

Since June 2012, the Dangme West District has been divided into the Prampram Ningo (the Coastal Zone) and Shai Osudoku (Dodowa/Forest and Osudoku/Lakeside Sub-districts) by legislative instrument.

1.8.3: Demography

There is a demographic and health surveillance system (DSS) in the district, which involves biannual visits to every household in the district to collect demographic data as well as data on migration and other health indicators. By December 2010, there were

22,767 households with 111,976 residents under surveillance. Forty point five percent (40.5%) of the population is below 15 years of age, which is not different from many other developing countries. Children aged below five years constitute 15.2% of the population with a sex ratio of 87 males to 100 females. Female headed households constitute 39.1%, this might be due to the fact more males compared with females migrate outside the district, usually for employment due to its closeness to major towns and cities such as Accra and Tema. The district population stood at 116, 288 at the beginning of 2012 (February) (Gyapong *et al*. 2013).

1.8.4: Economics

The district is very poor and typical of poor disadvantaged rural district across the country. Most of the inhabitants are subsistence farmers or fishermen. Other occupations are petty trading. There are a handful of trained artisans, craftsmen and a few civil servants, mainly migrant employees of government ministries, departments and agencies.

The widespread poverty in the district affects health. Poor health status and poverty are closely interrelated. Factors associated with living in poverty such as an unhealthy environment are the cause of much ill health and are compounded by ignorance, illiteracy, taboos and beliefs. The experience of ill health in turn exacerbates household poverty due to loss of income and the cost of health care. This situation results in a vicious cycle with poverty causing poor health and poor health maintaining people in poverty. Though the district holds most of the commercial cattle farms in the Greater Accra Region, this generally belongs to non-indigenes.

1.8.5: Climate

The district has two main seasons, the dry and wet. The major wet season starts from April to July and the minor from September to October. The rest of the year is dry, peaking in March.

1.8.6: Malaria Control in the district

1.8.6.1 Prevention

Distribution of Insecticide Treated bed Nets (ITN) free of charge to pregnant women and children under 5 years is done during the Maternal and Child health week (held once a year). Outside campaign periods, ITNs are sold at a subsidized rate to the same group of persons. ITNs are also distributed in the communities by non-governmental and community-based organizations. They are also on sale at pharmacies and chemical shops to the general public.

Intermittent Presumptive Treatment in pregnancy (IPTp) using Sulphadoxine Pyremethamine is done at all health facilities, which offer antenatal services, both public and private. In 2011, Indoor Residual Spraying was introduced in the district whiles all households were registered for free distribution of insecticide treated bed nets to household members in August 2012.

1.8.6.2 Prompt and accurate case management

Trainings are done for all prescribers on the management of malaria using ACTs and quinine. Follow up trainings are done annually. Manuals on the management of malaria have been distributed to all facilities and are available at the facility level. Three of the public sector facilities have microscopy for confirmation of malaria diagnosis. Laboratory staff are trained and retrained every year on the diagnosis of malaria at the laboratory.

Rapid Diagnostic Tests (RDTs) for malaria were introduced in August 2007 at the introduction of the RDT study in the District (Ansah *et al*. 2010). Subsequently, in 2009, the Ghana Health Service issued a circular for the use of RDTs in all facilities, which recommends and encourages its use especially at facilities where microscopy is not available. The tests kits are available at the Regional Medical Stores at no cost to primary public health facilities. ACTs are distributed to the facilities through the regional medical stores as well.

There is a Malaria Control Program point person at the District Health Administration who coordinates all the activities in the district. Monitoring of the various activities is done by the District, Regional and National health administration.

1.8.6.3 Malaria research in the Dangme West district

Various studies on malaria have been done in the district. Completed studies include:

- Mutual health Organizations (MHO's) in Ghana and implications for improving the success of health Insurance in Ghana.
- Perceptions of health providers on quality of care under the Dangme West district health insurance scheme.
- Malaria and access to health study
- Feasibility of deployment of Rectal Artesunate as pre-referral treatment for severe malaria in children under-5 years of age at the community level.
- Perceptions on and acceptability of Artesunate Amodiaquine for the treatment of malaria
- Home management of fevers (malaria and pneumonia) in children under-five: a cluster randomized controlled trial in southern Ghana.

- Rapid Testing for malaria in settings where microscopy is available and peripheral clinics where only presumptive treatment is available, a randomized control trial in Ghana.

Ongoing malaria studies at the time of study were:

- INDEPTH Effectiveness and Safety Studies of Antimalarial in Africa (INESS)
- Deployment of RDTs at chemical Sellers at the community level

1.9: Scope of Study

The overarching goal of the study was to determine the current levels of malaria transmission intensity in the Dangme West District of Ghana. Four specific project objectives that guided the research project were presented

1.10 Organization of Report

The report has been structured to reflect the different research objectives set out for this study. The research topic, Malaria; followed by the study background (with specific themes on the study area and population) are introduced to contextualise the study. The discussion in Chapter 2 responds to the literature review concerning the general topic of malaria, it's control and the need for the monitoring of malaria programs. It analyses the context, from global to National, then the finally to local context, in which the study is set. Different methodologies with their strengths and weaknesses, used for malaria transmission measurements are also presented. The research methodologies used in the study are presented in Chapter 3. Study results are presented in Chapter 4, first, results from the prevalence, followed by incidence and then entomology study. Chapter 5 discusses the research findings drawn all three studies. The report then concludes with a summary of the research outputs and study recommendations.

CHAPTER 2: LITERATURE REVIEW

2.1: Malaria

Malaria is a parasitic disease caused by protozoa of the genus *Plasmodium*, of the family *Plasmodiidae*, suborder *Haemosporidiidae* and order *Coccidia* (Warrell & Gilles 2002). Over 120 species occur in the blood of mammals and other animals and are mainly transmitted by *Anopheles* mosquitoes. In humans the transmission is exclusively by female anopheles mosquitoes. Human infections are caused by parasites from two subgenera, *Laverania* (*Plasmodium falciparum)* and *Plasmodium* (*Plasmodium vivax, Plasmodium ovale and Plasmodium malariae).* Four species, *falciparum, vivax, ovale, malariae* cause most of the human infections. *Plasmodium knowlesi* and other simian species (at least six species) are known to have caused zoonotic and accidental laboratory infections occasionally (World Health Organization 2013b; Roll Back Malaria 2008; Warrell & Gilles 2002; Kelly 1964)

The most severe form of disease is caused by *Plasmodium falciparum* (World Health Organization 2010b; Greenwood *et al*. 2008; Warrell & Gilles 2002), causing the majority of deaths and is also the main species in the tropical zones, including SubSaharan Africa, South East Asia, Western Pacific and the Amazon rain forest countries. *Plasmodium vivax* is the most widely distributed species and the main cause of the chronic disease forms. It is also the most predominant specie in Asia, Eastern Mediterranean, and the Amazon countries in the Americas.

The other three species of the *Plasmodium* occur less commonly but also cause infections in humans. *Plasmodium ovale* and *Plasmodium malariae* have inconsistent incidence and distribution generally. They occur in Sub-Saharan Africa, though their distribution is uncommon in Southeast Asia and Western Pacific regions. *Plasmodium malariae* and *Plasmodium ovale* infections make up a small fraction of the total pool of malaria infections and are limited in distribution to only Sub-Saharan Africa.

2.1.1 *Plasmodium falciparum*

Worldwide, the most pathogenic human infections are caused by *Plasmodium falciparum* and are associated with malignant, tertian or sub tertian malaria (Warrell & Gilles 2002; Kelly 1964). It has an average pre-patent period of 5.5 days an incubation period of 9-14 days and has been reported to cause epidemics. Its distribution is restricted to tropical and sub-tropical regions largely due to the fact that its development in the anopheles mosquito is impeded at temperatures below 20°C. It rarely causes relapses, but causes acute infections, developing quickly into severe and fatal forms of the disease in the non-immune, infants, young children and pregnant women. The severe forms of malarial disease associated with *Plasmodium falciparum* include cerebral malaria, algid malaria, malarial anemia, jaundice, renal dysfunction, malaria haemoglobinuria, hypoglycemia, pulmonary edema and haemostatic abnormalities. It also known to develop multiple widespread resistance to antimalarial drugs (Warrell & Gilles 2002; Kelly 1964).

2.1.2 *Plasmodium vivax*

Plasmodium vivax has the widest geographical distribution, occurring in tropical, subtropical and temperate regions outside Africa. It causes the most latent form of disease, and though less fatal, it causes the most debilitating form of disease (World Health Organization 2010c; Greenwood *et al*. 2008;Warrell & Gilles 2002). Polymorphism of its sporozoite is opined to explain this phenomenon. It is known to cause early infections in infants and young children, though adults also get severe clinical attacks. It frequently causes mixed infections with *Plasmodium falciparum* in tropical and subtropical zones. It has a pre-patent period of 8 days, with and incubation period of 12-17 days, though it may be prolonged to 8-9 months, especially in the temperate regions. It characteristically causes tertian malaria and requires a Duffy

negative blood group to invade red blood cells and as such does not infect Africans, as most of them lack the Duffy coat (Greenwood *et al*. 2008; Warrell & Gilles 2002). Acute mortality is low and cerebral form of disease has occasionally been reported in China (long form of incubation period, though mixed infection with *Plasmodium falciparum* was not ruled out). Severe forms of disease have been reported in the past and may be associated with other undercurrent diseases including malnutrition. It develops resistance to antimalarial drugs (White *et al*. 2014; Warrell & Gilles 2002).

2.1.3 *Plasmodium ovale*

Plasmodium ovale causes tertian malaria. It has a pre patent period of 9 days and incubation period of 16-18 days or longer. Its clinical manifestation resembles the disease form caused by *Plasmodium vivax* but spontaneous recovery is the norm and relapses are infrequent. *Plasmodium ovale* remains latent in the blood of humans in mixed infections mainly in Sub-Saharan Africa (occasionally reported in the West Pacific), suppressed by the more virulent *Plasmodium falciparum* or *Plasmodium vivax*, appearing and causing infections when the blood levels of the more virulent *Plasmodia* decline (White *et al*. 2014; Warrell & Gilles 2002).

2.1.4 *Plasmodium malariae*

Plasmodium malariae causes quartan malaria. It has the longest pre patent period of 15 days and incubation period of 18-40 days or longer. It occurs in patchy distribution over the same geographic distribution as *Plasmodium falciparum*. The disease forms resemble vivax malaria; occur in mixed infections with the more virulent falciparum and vivax. Though it has no latent hepatic forms, undetectable parasitaemia occurs with recrudescence, which can persist for decades as asymptomatic blood stage malaria infection and is usually detected during blood donation (Warrell & Gilles 2002).

2.2 Historical Perspective on Malaria Control and Eradication

Many global efforts have been made to control and eradicate malaria, from 1899 when Ross started anti-larval initiatives in Sierra Leone (Alonso *et al*. 2011;Warrell & Gilles 2002) By the mid-19th century, malaria was endemic in most countries and areas of the world, plaguing almost 90% of the entire population of the world, even to the Arctic Circle. Globally, 178 countries were endemic for malaria (Feachem *et al*. 2010; Roll Back Malaria 2008). Beginning in 1945, successful efforts to control malaria were achieved with Indoor Residual Spraying (IRS) with dichlorodiphenyltrichloroethane (DDT) (White *et al*. 2014; World Health Organiztion 2012; Warrell & Gilles 2002). In 1955 the 8th World Health Assembly launched the Global Malaria Eradication campaign for all countries with malaria except countries in Sub Saharan Africa and Madagascar, using IRS with DDT in combination with malaria case management. In 1957, the World Health Organization defined the concept of malaria elimination and its practice. In 1969, the Global Malaria Eradication Campaign was abandoned due to financial, technical and administrative issues (Feachem *et al*. 2010). By 1978, 37 of the 143 countries that were endemic in 1950 were freed from malaria (Baird 2010; Feachem *et al*. 2010), of which 27 were in Europe or the Americas. Malaria was successfully eliminated from the USA, Canada, Europe, and Russia. The Global Malaria Eradication effort paid off with major impact on malaria mortality and morbidity in most of the targeted countries but interruption of transmission was not achieved in some of the target countries. So in 1973 it was concluded that in some countries a "time-limited eradication program was impracticable". By 1979, the WHO developed and shifted into the strategy of long-term integrated control programs and the Global Malaria Eradication campaign was abandoned. Even after the abandoning of the global eradication effort, some countries

have successfully eliminated malaria since that period (Tunisia (1979), Maldives (1984), United Arab Emirates (2007) Morocco

(2010) Turkmenistan (2010) Armenia (2011).)(Alonso *et al*. 2011; World Health Organization 2011; Roll Back Malaria 2008). By the year 2010 worldwide, 99 countries were still malaria endemic, out of which 67 were in the control phase with 32 in various stages of elimination (Feachem *et al*. 2010).

In 1985, the 38th World Health Assembly recommended the integration of malaria control efforts into national primary health care systems in malaria-affected countries. By 1991, strategies for main prototypes of malaria control had been developed, and the World Health Organization Global Malaria Control Strategy was approved and adopted in 1992 by the ministerial conference on malaria. In 1998, the Roll Back

Malaria Partnership was formed to coordinate global efforts in fighting malaria (Roll Back Malaria 2008; Warrell & Gilles 2002).

2.3 Malaria Epidemiology

Human Malaria infections has been reported from all latitudes, above and below sea level throughout the world (Warrell & Gilles 2002). It occurs in focal areas largely due to the fact that its transmission depends on environmental and other factors (White *et al*., 2014; Roll Back Malaria, 2008). Transmission occurs when an infected female Anopheles mosquito bites a human being (victim). The source of infection is usually a sick or symptomless malaria parasite carrier human being (donor), so the chain of transmission is completed when a female anopheles mosquito transmits the disease from an infected human carrier to the human victim (World Health Organiztion 2013). Sporadically, malaria parasites are transmitted through infected blood transfusion and infected hypodermic needle use. Malaria parasites require two hosts for the completion of their life cycles, the intermediate host (usually mammalian, in which asexual

development occurs) and the definite host (anopheles mosquito, in which sexual reproduction happens). The life cycle alternates between the two hosts. Taxonomically, they are associated with two types of asexual division, schizogony in the mammalian host and sporogony in the insect vector. The Plasmodia replicate quickly and extensively through three vegetative stages, in the vertebrate host, within red blood cells (erythrocytic) and other tissues (preerythrocytic or exo-erythrocytic).

The female anopheles mosquito must take blood meals every 2-4 days to support successive development of eggs. On biting an infected mammalian host, asexual schizogonic stage and gametocyte (gamete forming first sexual stage) forms of the *Plasmodium* are taken with the blood meal. Once in the gut of the mosquito, the gametocytes are activated by lower temperatures in the region of 5°C to develop within the mosquito gut wall into the female and male gametes (White *et al*. 2014; World Health Organiztion 2013a). They then fuse to form the zygote, which undergoes further development into an oocyst. The oocyst undergoes further transformation in the gut wall of the mosquito and bursts to release sporozoites about 10-15 days later into the salivary gland of the anopheles mosquito. The completion of the life cycle of the malaria parasite in the mosquito, from the time of ingestion of the infected blood meal to the transmission into the human hosts usually takes 7-21 days, depending on species and the ambient temperature and humidity (White *et al*. 2014; Warrell & Gilles 2002). The infected female *Anopheles* mosquito bites a human being and injects sporozoites with saliva (used as an anticoagulant) into the blood stream.

The saliva prevents the blood from clotting in the mosquito's proboscis. Once inside the human blood stream, sporozoites move quickly into the liver and attempt to invade the liver cells. Once they succeed, the parasites divide and generate many thousands of new parasites over a period of 7–21 days. An enlarged liver cell, filled with replicating

parasites called a liver schizont, finally bursts, releasing thousands of merozoites into the bloodstream, which quickly attach to and invade red blood cells. On entering a red blood cell, the malaria parasite begins to mature, transforming into a trophozoite (World Health Organiztion 2013a ;Warrell & Gilles 2002). This is the normal liver phase for *Plasmodium falciparum* and *Plasmodium malariae*. In *Plasmodium vivax* and *Plasmodium ovale*, a number of the parasites that originally invaded the liver cells do not immediately become liver schizont but enter a latent phase, called hypnozoites. These dormant parasites cause relapses of clinical disease at intervals after the first clinical malaria attack (White *et al*. 2014; Warrell & Gilles 2002). The liver stage of malaria parasite infection is symptomless and lasts for about six days, with each sporozoite producing tens of thousands of merozoites, which then invade and develop within erythrocytes.

The blood stages of infection begins with asexual forms of the parasite that go through repeated cycles of duplication, whiles some differentiate into male and female gametocytes (sexual forms), which await ingestion by mosquitoes before further development. Asexual blood stage parasites produce eight to twenty new merozoites every forty-eight hours (seventy two hours for *Plasmodium malariae*), leading to rapid rise in parasite number levels over a thousand merozoites per erythrocyte. The asexual stages are pathogenic, causing diverse sequelae in different organ systems in the infected individual. Sexual stage parasites are nonpathogenic but are transmissible to the female *Anopheles* mosquito vector, in which they combine to generate genetically distinct sporozoites making the mosquito vector infectious to its next blood meal donor almost two weeks after ingesting gametocytes. The development of *Plasmodium vivax* within the mosquito vector can occur at a lower environmental temperature than that necessary for the development of *Plasmodium falciparum* (Warrell & Gilles 2002).

2.3.1 Transmission Intensity and Classification

Malaria trans mission (of parasites) intensity varies geographically (Warrell & Gilles 2002; Roll Back Malaria 2008). It affects all aspects of the epidemiology of malaria including community prevalence (including age-specific prevalence of infection), disease incidence (including the mix of types of disease syndromes) and disease mortality (both community and health facility based). Its effect on disease epidemiology therefore makes it the key determinant of the expected outcomes of malaria control efforts. Malaria transmission intensity is determined mainly by the length of life, density, biting habits, and efficiency of the mosquito vector (Greenwood *et al*. 2008). Twenty out of the over four hundred species of anopheles mosquitoes are efficient vectors of malaria (Warrell & Gilles 2002) The most effective vectors are human biting, live long, breed eagerly, occur in abundance and are largely unaffected by environmental change (White *et al*. 2014).

Malaria transmission can be described as high, moderate or low, stable or unstable. It can be endemic or non- endemic. Malaria endemicity can be described as holo, hyper, meso or hypo endemic. All year round malaria infection is said to be stable transmission. Transmission is termed unstable where there are large variations in malaria transmission over seasons and years. The entomological inoculation is less than five (sometimes one) infective bites per person per year and impedes the development of immunity. Full protective immunity from malaria is therefore not acquired and acute symptomatic disease can occur at all ages, which progresses quickly to severe disease if not treated promptly and adequately (World Health Organization 2010b). In such areas, changes in environmental, economic, or social conditions, heavy rains after drought, large population movements together with a breakdown in malaria control and prevention services (often because of armed conflicts) can result in epidemics, with

substantial mortality in all age groups. Malaria transmission is characteristically unstable and uncommon in most areas of low transmission (Greenwood *et al*. 2008). High Malaria transmission is usually used to describe hyper-endemic and holo-endemic malaria. Holo-endemic malaria transmission is usually used to describe areas with parasite prevalence in children aged 0-11 months of age of over 75%; the transmission is intense, perennial and produces significant immunity to malaria disease immediately after early childhood. Hyper-endemic malaria transmission is usually used to describe areas with parasite prevalence in children aged 2 to 9 years of over 50%. The transmission is intense but seasonal and immunity to malaria disease is inadequate in all age groups. Moderate transmission refers to meso-endemic malaria, which corresponds to areas with 11 to 50% parasite prevalence among children aged 2 to 9 years, with wide geographical variations in risk of malaria transmission. Hypo-endemic malaria refers to areas with parasite prevalence less than10% of parasite prevalence in children 2 to 9 years of age, but may be higher for part of the year. Overall, malaria transmission is minimal (Warrell & Gilles 2002; Kelly 1964).

The thresholds stated above are not, fixed, and surveillance strategies have to be set according to program goals, local conditions and needs. In most of Asia, South and Central America, where transmission is largely low and seasonal and unstable, most inhabitants receive one or fewer infectious mosquito bites per year. Malaria transmission intensities are much higher in most regions of sub-Saharan Africa, where *Plasmodium falciparum* forms the major parasite and in parts of Oceania. The entomological inoculation rates in these parts of sub Saharan Africa may be as high as a thousand infective bites per person year, with attendant high malaria morbidity and mortality mostly prominent during early childhood. From childhood, they graduate into asymptomatic carriers of malaria infection by the time they reach early adulthood.

Unstable malaria transmission also refers to low, erratic or focal malaria transmission (Coulibaly *et al*. 2014; World Health Organization 2012b; Cairns, Rocafeltrer, *et al*. 2012; Roll Back Malaria 2008). Full protective immunity from malaria disease does not develop in individuals living in areas of unstable malaria transmission. Full-blown malaria infections can occur in all age groups.

Significant variation in environmental, economic, or social conditions such as in large population movements following conflicts, disasters, droughts or changes in climatic conditions such as heavy rainfall after drought, large population movements with resultant breakdown in health and malaria infrastructure easily result in malaria epidemics. These epidemics result in significant malaria mortality in all age groups (World Health Organization 2000).

2.3.2 Prevention of Malaria

Effective tools for the prevention of malaria exist for most settings, for all ages with the potential of reducing morbidity and mortality. The basic preventive tools are vector control and chemo prevention/prophylaxis (Roll Back Malaria 2008).

2.3.2.1 Chemoprevention and chemoprophylaxis

- Chemoprophylaxis is recommended for non-immune travelers who travel to malaria endemic areas with potential of exposure to malaria.

  Chemoprophylaxis is recommended in combination with the use of protective measures such as the use of ITNs, protective clothing and the use of insect repellants (Neave *et al*. 2014; Wieten *et al*. 2013).
- Intermittent preventive treatment with Sulphadoxine–Pyremethamine for pregnant women (IPTp). The recommendation is for every pregnant woman in *Plasmodium falciparum* endemic areas to receive a minimum of three doses of

Sulphadoxine–Pyremethamine. Growing resistance to Sulphadoxine–Pyremethamine in some regions has arisen (World Health Organization 2013b).

- Seasonal Malaria Chemoprevention (SMC) for *Plasmodium falciparum* malaria control in children aged three to fifty nine months in the highly seasonal malaria transmission areas of the Sahel sub-region in Africa (World Health Organization 2012b; Cairns, Roca-Feltrer, *et al*. 2012).

2.3.2.2 Vaccination

Development of malaria vaccines has taken a lot of effort and resources. Currently the RTS, S vaccine, which targets the circumsporozoite (CSP) protein of *Plasmodium falciparum*, is the most advanced in development (Abdulla *et al*. 2013).

2.3.2.3 Mosquito vector control

The recommended tools currently in use work by reducing human mosquito contact and by decreasing the lifespan of the mosquito vector**.** The most current, recommended and most broadly applied tools are:

- Insecticide Treated nets (ITNs), which include long-lasting insecticidal nets (LLINs).
- Indoor residual spraying (IRS).
- Larval control (including environmental management) for settings where the mosquito vector breeding sites are few, fixed, findable, easy to identify, map and treat.

Insecticide resistance affecting all the main classes of insecticides and vector species has been the major problem for vector control efforts. Currently, resistance has been detected in 64 countries with ongoing malaria transmission (White *et al*. 2014; World Health Organization 2013; Moss *et al*. 2012).

2.3.3 Treatment of Malaria

Combination therapies are recommended for the treatment of all forms of malaria. . Monotherapy of Artemisinin or any the partner drugs for the treatment of malaria should not be done (World Health Organization 2010b).

2.3.3.1 Treatment of falciparum Malaria

Artemisinin-based combination therapies (ACTs) are the recommended treatments for all forms of falciparum malaria. The choice of ACT in any country will be founded on the level of resistance of the partner medicine in the combination in the region.

For uncomplicated *Plasmodium falciparum* malaria recommended treatments are:

- Artemether plus Lumefantrine
- Artesunate plus Amodiaquine
- Artesunate plus mefloquine
- Artesunate plus sulfadoxine-pyrimethamine.

Second-line antimalarial treatment for uncomplicated *Plasmodium falciparum* must be known to be efficacious effective in the country and region. These include:

- Artesunate plus tetracycline or doxycycline or clindamycin
- Quinine plus tetracycline or doxycycline or clindamycin.

2.3.3.1.1 Treatment of Malaria in Pregnancy

In the first trimester, the recommended drugs are:

- Quinine plus clindamycin or Artesunate plus clindamycin
- Or country or region specific recommended ACT

In the second and third trimesters, the recommended drugs are country or regional recommended ACTs or Artesunate plus clindamycin or quinine plus Clindamycin.

For lactating women, country or regional recommended ACTs are sufficient but should not be given any combination containing dapsone, primaquine and tetracyclines.

2.3.3.1.2 Treatment of Imported falciparum malaria

Recommended treatments for imported malaria in non-endemic countries for *falciparum* malaria are:

- Atovaquone-proguanil;
- Artemether-Lumefantrine;
- Quinine plus doxycycline or clindamycin.

2.3.3.1.3 Treatment of severe malaria

In both children and adults, intravascular (IV) or intramuscular (IM) Artesunate is recommended.

Parenteral artemether or quinine can be used in the absence of parenteral Artesunate. Parenteral antimalarial must be given for at least twenty-four hours once started irrespective of the patient's ability to tolerate oral medication at diagnosis. This treatment should then be augmented with a complete course of an ACT, Artesunate plus clindamycin or doxycycline or quinine plus clindamycin or doxycycline. Recommended pre-referral treatment for severe malaria includes rectal Artesunate, intra muscular quinine, intramuscular Artesunate or intramuscular artemether.

2.3.3.1.4 Treatment of Plasmodium vivax Infections

For Chloroquine-sensitive *Plasmodium vivax* malaria infections, Chloroquine plus primaquine is the treatment of choice in mild-to-moderate G6PD deficiency, primaquine 0.75 mg base/kg body weight once a week for 8 weeks is recommended. In severe G6PD deficiency, primaquine is contraindicated. With the exception of Artesunate plus SP (it is ineffective against vivax malaria), ACTs (adopted as the first-

line treatment for *Plasmodium* falciparum malaria) plus primaquine may also be used for the radical cure of *Plasmodium vivax* malaria (Price 2014; World Health Organization 2010b).

2.4 Monitoring and Evaluation of Malaria Programs

Monitoring and Evaluation including surveillance of Malaria Programs is vital for optimal performance of programs (Alonso n.d.).

Monitoring is the regular tracking of the key parts of program project over the project lifetime, usually at the inputs and outputs level to ascertain whether implementation is on track, whiles, **Evaluation** is the systematic collection and analysis of information about the characteristics of targeted results or outcomes of a program or project (World Health Organiztion 2013b; World Health Organization 2012a; Carneiro Ilona &Howard Natasha 2011).

Current recommendations are that monitoring and evaluation of malaria control efforts be done through health information systems (disease surveillance), household surveys, health facility surveys and Demographic Surveillance Systems (Malaria Policy Advisory Committee Meeting 2014; World Health Organization 2012a).

2.4.1 Malaria Surveillance

Robust malaria surveillance systems are critical for program design, implementation, monitoring and evaluation (Bousema *et al*. 2012; Hay *et al*. 2010; Gemperli *et al*. 2006). They are very useful for the targeting of scarce resources to the populations and areas most in need, to forecast epidemics and to respond to unusual trends (outbreaks, epidemics, poor response of case load or to widespread implementation of interventions) (World Health Organization 2012a). They are an easy and available source of data for monitoring control, though it's use for the estimation of malaria

transmission is unreliable if the system collects data only from health facilities (Satoguina *et al*. 2009). Its design mainly depends on malaria transmission levels and the resources available for surveillance, the best designs incorporate both health systems data with data from other sources such as population and other transmission measurement studies (Bretscher *et al*. 2012). It is recommended for surveillance systems to be integrated into national health information systems or Communicable disease surveillance system. In moderate to high transmission countries in malaria control mode, may not need to examine and react to every confirmed case individually. Emphasis is placed on aggregation of case data and actions taken at the population level (community). As transmission decreases to low transmission levels, it then becomes essential and feasible to monitor and react at each individual case level. When programs transition from control to elimination phase, surveillance systems need to be set to detect every malaria infection, symptomatic or not, and investigate each case to determine the source of infection for appropriate and adequate remedial measures to be effected (Bousema *et al*. 2012; World Health Organiztion 2012).

2.4.1.1 Malaria surveillance in Moderate to high transmission settings

Data should be continuously, systematically and routinely collected and analyzed at health-facility, district, and other higher administrative as well as at the National levels in order to set priorities for malaria control activities. Registers are expected to be kept at health facilities on all individual outpatient cases with type of diagnostic tests performed with results, which are then aggregated and reported to the district and higher levels subsequently. Case based surveillance is recommended for all severe disease cases and inpatient cases and deaths and analyzed monthly to asses control efforts at health facility levels. At district and national levels, cases and deaths are analyzed monthly on control charts for trend identification, evaluate interventions and respond

appropriately (World Health Organization 2012a). The five areas covered by the control charts are:

- malaria incidence and mortality rates,
- proportional malaria Incidence and mortality rates,
- general patient attendance rates,
- diagnostic activity (annual blood examination rate), Quality of diagnosis and health-facility reporting.

2.4.1.2 Malaria surveillance in low-transmission settings

Registers of individual malaria cases are expected to be maintained at health facilities, with the type of diagnostic tests done and test results obtained recorded. Aggregate data of both outpatient and inpatients are to be reported to district and higher administrative levels, with line lists of inpatients malaria cases and inpatient malaria case outcomes especially malaria deaths, are to be reported to the district and subsequent levels monthly.

At the district level, malaria cases and deaths are summarized weekly or monthly on the same five control charts used in high transmission settings, to assess the impact of malaria control interventions and identify trends that require urgent response. Analysis of the data is encouraged to be done by health-facility catchment area and by village or community, to review and set priorities for activities. A register of severe cases and deaths should be maintained and investigations are encouraged to identify and address program weaknesses when necessary. At national level, cases and deaths are summarized monthly on the five control charts, to assess the impact of malaria control interventions.

2.4.1.3 Malaria surveillance systems in the elimination phase

Case-based surveillance is carried out and each confirmed case is immediately notified to district, provincial and central levels. A full investigation of each case is undertaken to determine whether the infection was imported, acquired locally by mosquito-borne transmission (introduced, indigenous or relapsed) or induced. The national reference laboratory re confirms all positive test results and a sample of negative test results, leads the national laboratory quality-assurance (QA) program.

2.4.2 Malaria Household Surveys

Malaria Household Surveys focus on measuring key malaria indicators through nationally representative household surveys (Malaria Initiative 2013). Currently, the main nationally organized household surveys are;

1. Malaria Indicator Surveys (MIS), these measure indicators set by the Roll Back Malaria, Global Malaria Action Plan, the President's Malaria Initiative and the Millennium Development Goal. Data collected cover ownership and use of insecticide-treated mosquito nets, indoor residual spraying with insecticides, prompt and effective treatment of fever in children under five years of age, and prevention of malaria in pregnant women (IPTp and ITN use). Recently, biomarker tests for malaria and anemia have been included in the Malaria Indicator Surveys.
2. Demographic and Health Surveys are nationally representative household surveys that make available information for a broad array of data for monitoring and impact evaluation on population, health, and nutrition indicators (ICF International 2012). There are two types of surveys:

- Standard Demographic and Health Surveys which cover very big sample sizes in the range of 5,000 and 30,000 households and are carried out at five year intervals, to allow for comparisons over time.
- Interim Demographic and Health Surveys focuses attention on the collection of data on key performance monitoring indicators but may not cover data for impact evaluation indicators. They are surveys are done between rounds of Demographic and Health surveys. They are nationally representative, have smaller sample size and also have shorter questionnaires than the Demographic and Health surveys.

On malaria specific indicators, the Demographic and health surveys since 2000 collects data on ownership and use of mosquito nets by children and pregnant women, prevalence and prompt treatment of fever in children under five years, and coverage of intermittent preventive treatment of malaria in pregnant women. Questions on indoor residual spraying, biomarker testing for anemia and malaria, type and timing of antimalarial drugs have recently been added. Data is also collected on prevalence of anemia and iron supplementation (ICF International 2012).

2.4.3 Malaria Health Facility Surveys

These are done to monitor diagnostic testing and treatments (adherence to treatment guidelines) at health facilities.

2.4.4 Demographic *Surveillance Systems*

Demographic Surveillance systems are set up primarily to define the risk and corresponding dynamics in birth, mortality and migration rates in a defined population over time. Currently present in Africa, Asia and Latin America, they can be used to monitor trends in malaria transmission (Baiden *et al*. 2006).

2.5. Malaria Transmission Measurement Methods

Measurement of malaria transmission is key in tracking progress in malaria control efforts (Cook *et al*. 2010; Drakeley *et al*. 2005), Though various methods are employed in the measurement of malaria transmission intensity, there is lack of consensus among the malaria science community as to is the which method is the best and most efficient (Kelly-Hope & McKenzie 2009).

National Malaria Indicator Surveys (MIS) as well Multiple Indicator Cluster Surveys (MICS, a UNICEF child national child indicator survey with malaria indicators, which measures parasite prevalence are used to track milestones for endemic countries strive at achieving control goals in high to moderate transmission countries.
(Chan *et al*. 2010; Steketee & Campbell 2010; Nahlen & Low-Beer 2007).

2.5.1. Entomological Inoculation Rates

Traditionally, malaria transmission has been measured by entomological studies, which measure the pressure of transmission. (Beier *et al*. 1999), and is a key measurement of the extent of exposure of the human population in a given area to malaria (Smith *et al*. 2004). Entomological studies have been the gold standard for measuring malaria transmission, but the cost of this can be very expensive (O'Meara,
W. P., Collins, W. E., & McKenzie 2007; Drakeley *et al*. 2005; Snow *et al*. 1996; Pull, J. H.; Grab 1974). It estimates the transfer of malaria parasites from infective anopheles species to humans. It can be defined as the number of infective bites an individual receives per unit (Kelly-Hope & McKenzie 2009; Schellenberg *et al*. 2003; Beier *et al*. 1999). Beier *et al* also showed a direct correlation between the level of exposure to infective bites (EIR), asexual erythrocytic stage parasite density and the burden of disease at the community level (Beier *et al*. 1999). The level of exposure to anopheles

infective bites is an important variable therefore for the determination of the burden of malaria in any community. The EIR had proved to be a more instant measure of transmission intensity than other measures, such as malaria prevalence, incidence or hospital-based measures of infection or disease incidence (Kelly-Hope & McKenzie 2009; Beier *et al*. 1999). It is a good indicator of malaria transmission (Robert *et al*. 2003) at high mosquito density. At low densities (below entomologic threshold of detection), EIR estimation becomes logistically challenging and problematic, though transmission still occurs (Kusi *et al*. 2014; Yukich *et al*. 2012).

The EIR is influenced by several factors that include vector species, vector bionomics, feeding preferences, resting behavior, vector numbers, distribution, genetic polymorphism and vector efficiency among others (Corran *et al*. 2007; Drakely *et al*, 2005; Beier *et al*. 1999). It is also not an exact measure, as not all bites from infective mosquitoes result in transmission. Entomological Inoculation Rate (EIR) uses human blood indices, sporozoite and human biting rates to estimate transmission (Snow *et al*, 1996). With effective roll out of malaria interventions aimed at controlling the burden of malaria, the precision of estimating transmission by EIR methodology has been questioned due to its lack of precision, uneven distribution of mosquitoes in communities and low sporozoites rates even in endemic areas (Drakeley *et al*. 2005; Snow *et al*. 1996). The time and logistical challenge of mounting intensive entomological surveillance needed for the calculation of entomological rates is also very considerable and makes it laborious (Kusi *et al*. 2014; Snow *et al*. 1996; Pull *et al*. 1974)

2.5.2. Parasite Prevalence

The commonest measured metric of malaria transmission intensity is the malaria parasite prevalence rate. Also known as Parasite Rates, (PR), they are generated

through community surveys, and forms the bulk of information available worldwide on monitoring and evaluating malaria endemicity (Moyes *et al*. 2013). It is the proportion of a group of individuals infected at a given point in time (Gething *et al*. 2011; Hay *et al*. 2008). Malaria parasite prevalence measurements are very rapid, easier to do, relatively economical, less time consuming than incidence and entomologic measurements and levels for specific age groups are used to define endemicity (Satoguina *et al*. 2009; Corran *et al*. 2008; Hay *et al*. 2008). It provides a more specific estimate for malaria infection (Hay *et al*. 2008). Changes in parasite prevalence over time can be a more exact and useful tool in tracking changes in incidence burden over time, though it cannot be said to correspond with decreases in severe malaria and deaths burden (Greenwood & Koram 2014). Its precision depends largely on the burden of malaria infection, the sample size and the diagnostic test used (Gething *et al*. 2011; Hay *et al*. 2008). However, in the interpretation of the results, there is difficulty in matching present parasite status and disease burden and outcomes

(Burkot & Graves, 1995). It is also insensitive especially when microscopy is used (Kusi *et al*. 2014) and poorly defines endemicity, as a specific level can exist as a result of a wide ranges of EIRs, depending on mosquito species, densities , biting patterns, duration of infection and community treatment behaviors (as well as patterns) (Yukich *et al*. 2012). The results may also be seasonal (Satoguina *et al*. 2009). The sample size needed to accurately estimate prevalence or changes in prevalence also increases significantly as transmission decreases.

2.5.3. Incidence Rates

Malaria Incidence is the rate of diagnostically verified clinical malaria in a population within a defined time period. It is the Incidence of new malaria infections and is also referred to as the force of malaria infection (Yukich *et al*. 2012). It may be measured

passively or actively (Schellenberg *et al*. 2003). It demands the diagnosis of every suspected malaria case, usually through a systematic surveillance system. It can be done by passive or active case detection. Passive case detection involves the examination of suspected mostly febrile cases that present routinely at health facility service points for care, and it is the easiest way to estimate malaria incidence. Passive case detection depends on documentation of malaria episodes presenting voluntarily at health facilities, thus depending heavily on health seeking behavior of inhabitants of the locality. It is the most easily accessible longitudinal data for tracking and monitoring malaria transmission. Though it produces no information on sub-clinical infections, treatment methods used at the household level and home treated infections are generated, which are very useful for intervention targeting especially as transmission decreases to lower levels (Yukich *et al*. 2012).

In active case detection persons are visited regularly over a period of time at home. It therefore describes age and temporal distribution of disease without the influence of health seeking behavior (Schellenberg *et al*. 2003). It also allows for the identification and treatment of asymptomatic carriers, who form the source or pool of infection for continuous malaria infection at the community level (Feachem, 2009), and is a very important tool as malaria control programs transition into elimination. It reflects the intensity of transmission. It is most useful in the description of age patterns of morbidity and mortality, thus determining which age group or locality at which interventions need to be targeted. It requires multiple visits to households over a period of time, making it neither feasible nor cost effective for routine monitoring of malaria transmission (Yukich *et al*. 2012). The results are usually expressed as malaria incidence per 1000 of the population of the area it represents.

2.5.4. Emerging Transmission Measurement Methods

Many studies have suggested and shown that antimalarial antibody prevalence could give better estimates of transmission intensity than the traditional EIR and parasite prevalence rates. These studies also proposed the relative ease and accuracy of using parasite and serological surveys to estimate the force of malaria transmission (Kusi *et al*. 2014; Badu *et al*. 2012; Yukich *et al*. 2012; Noor *et al*. 2011; Cook *et al*. 2010; Williams *et al*. 2009; Drakeley & Reyburn 2009; Corran *et al*. 2007; Drakeley *et al*. 2005).

2.5.4.1 Uses of Emerging Transmission Methods in Field Malaria Transmission studies

Drakely *et al* (2005) conducted two age-stratified randomized cross-sectional surveys of 250 people in each of 12 villages in three altitudes transects in the North Pare, South Pare, and Western Usambara mountains of north-eastern Tanzania, during which serum samples were collected. The samples were analyzed and reported as

Sero-prevalence (defined as the proportion of participants whose serum gave an ELISA value above the normal range for serum from malaria non-exposed European individuals). The data was then analyzed using reversible catalytic model. The mean annual rate of conversion to Sero-positive, the mean annual rate of reversion from Sero-positive to Sero-negative, were fitted into the model using maximum likelihood assuming a binomial error distribution

The equation used for each village was:

$$Pt = \frac{\lambda}{\lambda+\rho}(1 - \exp(-(\lambda + \rho)t)$$

Where:

Pt = proportion of Sero-positive participants aged *t*

λ = village-specific annual rate of Sero-conversion (negative to positive)

ρ = village-specific overall annual rate of Sero- reversion (positive to negative)

The results showed that sero-prevalence reflected cumulative exposure and therefore with malaria prevalence data can be used to estimate malaria transmission over time (Drakeley *et al*. 2005).

In their review, Corran *et al* (2007) suggested that the ideal tool for determining malaria endemicity should integrate malaria exposure or infection over time.

Individuals exposed to malaria infection may remain sero-positive for antimalarial antibodies for long periods of time though the relevance and interpretation of persistence of these antibodies is still being debated (Corran *et al*. 2008). The review listed various methodologies that have been used to estimate malaria endemicity over time. Discrediting the use of immune-fluorescence, which had limited use because of its reliance on cultured parasites and fluorescence microscopes, as well as malaria microscopy with its subjective nature of the slide readings, the paper recommended ELISA measurements, which are standardized and are relatively easy to do and its availability.

Using ELISA measurements of antimalarial antibodies has also been shown to be a potentially useful epidemiological tool (Drakeley & Cook 2009) but lacks standardization (Drakeley *et al*. 2005), but modeling age specific malarial antibody prevalence data into reversible catalytic model gives good estimates comparable to EIRs (Bosomprah 2014; Drakeley & Cook 2009; Drakeley & Reyburn 2009)In their systematic review to confirm the methodology for stabilizing antibodies stored in dried blood spots under different conditions, Williams *et al* confirmed that dried used RDTs could be stored at or below 4°C for long periods when desiccated with silica gel, (Williams *et al*. 2009). Recovery of antibodies at this condition was similar for plasma

and desiccated dried spots (filter paper). At ambient temperature, spots and antibodies were stable for very limited time periods. Antibodies eluted from dried spots were compared with plasma values in samples collected from North Eastern Tanzania and two localities in Uganda. Paired dried spot and serum samples were taken from participants visiting dispensaries in Tanzania and health facilities in Uganda from finger pricks. Those with active malaria infection were excluded.

A cross sectional serological survey to monitor changes in malaria transmission was conducted in Vanuatu in 2009 to assess the role of the use of serological markers in the evaluation of malaria transmission (Cook *et al*. 2010). Two of the southernmost islands of the Vanuatu archipelago, Aneityum and Tanna where malaria transmission intensity has been reduced following control measures, and looking forward to elimination were chosen as study sites.

Using serological markers for exposure to *P. falciparum* and *P. vivax*, filter blood spot papers were collected from 517 participants from Aneityum and 1,249 from Tanna to assess the prevalence of antibodies to MSP-1_{19} and AMA-1and *P. vivax* MSP-1_{19} and AMA-1. They then modeled age-specific Sero-prevalence using a simple catalytic conversion model based on maximum likelihood to generate a community seroconversion rate. Their results distinguished between areas of differing domination by P. *vivax* or *P. falciparum.* Age-stratified results also showed a decrease in Seroprevalence, which occurred about 30 years ago in both study islands, which may indicate a change in transmission intensity in that period of time. It was suggested that several children in Aneityum might have been exposed to malaria since the 2002 *P. vivax* epidemic. Subsequently, similar methods have been used in monitoring changes

in transmission from mainly low transmission settings, including Cambodia (Cook *et al*. 2012).

2.5.5. Malaria Diagnostic Tests

Malaria diagnosis is suggested by presenting clinical symptoms and signs and confirmed by laboratory testing of evidence of malaria parasites in peripheral blood.

The current recommendation is for all diagnostic tests to be quality assured (World Health Organization 2013b; World Health Organiztion 2013).

2.5.5.1 Malaria Microscopy

Microscopy remains the certain and "gold standard" for the diagnosis of all human forms of malaria. Like many laboratory dependent diagnostic tests, it requires highly trained and supported staff, laboratory space, a working microscope and electricity (Drain *et al*. 2014; White *et al*. 2014; All-Parliamentary Group on Malaria and Neglected Tropical Diseases 2011; Warrell & Gilles 2002). Malaria microscopy is also subjective in the nature of the slide readings needing specialist slide readers (Corran *et al*. 2007). It is invasive, logistically demanding (capital requirement and dependence on electricity) and the risk estimates it provides varies seasonally (Hay *et al*. 2008). Results from microscopy is subjective, depending largely on the skills of the reader. The sensitivity also becomes limited in low density malaria infections (Okell *et al*. 2009).

2.5.5.2 Malaria Antigen Detection Tests (Rapid Diagnostic Tests)

Malaria Rapid Diagnostic Tests (RDTs) have been used in malaria diagnosis at health facilities to diagnose malaria (augment microscopy) (Ilombe *et al*. 2014; Bastiaens *et al*. 2014; Tiono *et al*. 2014; Samadoulougou *et al*. 2014; Ansah *et al*. 2010; Laurent *et al*. 2010). They are also used in malaria case investigation and surveillance (Cheng *et*

al. 2014; Tiono *et al*. 2014) Its development has been a key advancement in the attempt to scale up parasitological diagnosis of malaria, mainly at health facilities (Cheng *et al*. 2014; Wongsrichanalai *et al*. 2007; World Health Organization 2006). RDTs are expected to have high sensitivity to be able to detect all clinical infection and high specificity to be used in monitoring in low prevalence areas for them to be useful (World Health Organization, FIND 2012). The current WHO recommendation is that every case of malaria be confirmed parasitologically by microscopy or RDT before treating with ACTs.

RDTs are lateral flow devices which use antibodies present in a small quantity of blood to capture and detect malaria parasite antigen by immuno-chromatography (Cheng *et al*. 2014; World Health Organization *et al,* 2007). They detect malaria antigen in a small amount of blood (5–15 µl) by immuno-chromatographic assay with dye labeled monoclonal antibodies (impregnated on a test strip) directed against target parasite specific antigens in lysed blood. The dye-labeled antibody on the strip/cassette binds to the parasite antigens, forming a complex, which gets captured on a nitrocellulose strip by a band of bound antibody, forming a visible line called the

"capture" or "test" line. The result, when positive is usually a colored test line and develops within 5–20 minutes. (World Health Organization 2006; World Health Organization, FIND 2012). The second line in the kit window is the control line which does not confirm the antigen testing ability of the kit, though it's presence gives indication to the integrity of the bound dye-antibody complex (Tiono *et al*. 2014; USAID Quality Assurance Project, University Research Co. 2009). RDTs have neither need for capital investment (microscopes) nor electricity. They are also easy to perform and interpret. Present day RDT test formats encourage user friendliness and safety as compared to earlier forms. The most common RDTS in use detect only *Plasmodium*

falciparum though those that differentiate between the three nonfalciparum species are accessible.

Currently, RDTs detecting malarial antigen histidine-rich protein-2 (HRP-2) and malaria parasite species specific or across species lactate dehydrogenase (pLDH) or aldolase antigens have been commercialized and available for testing in many areas (White *et al*. 2014; Warrell & Gilles 2002). Studies so far, have shown that the sensitivity can be as high as, or even higher as expert microscopy (Drakeley & Reyburn 2009) though Tiono *et al* concluded in their study that RDT sensitivity in field trials decreased with age (Tiono *et al*. 2013).

Other studies in East Africa have also shown that RDT derived immunoglobins can give results as accurate as traditional filter paper eluates, with the dual advantage of giving rapid, easy to use means of malaria diagnosis in the field as well (Corran *et al*., 2007; Drakeley & Reyburn 2009; L. Stewart *et al*. 2009; *P*.).

It's use in field population malaria epidemiological studies has been limited, prior to 2010, but since then, it's use in Demographic and Health Surveys has been reported in Burkina Faso (Samadoulougou *et al*. 2014) and in the Multiple Indicator Cluster Survey in (Ghana Statistical Service 2012) among children under five years of age.

2.5.5.2.1 HRP-2 detection RDTs

Generally, HRP-2 RDTs have higher sensitivity (95%) at parasite density 100 parasites/µl and are more stable thermally than the non- HRP-2 RDTs (Cheng *et al*. 2014; World Health Organization, FIND 2012). Extensive variation in antigenic expression of HRP-2 by *P. falciparum* is a drawback. There are also regions where *Plasmodium falciparum* parasites do not express HRP-2 antigens, making them useless

for testing (Samadoulougou *et al*. 2014; World Health Organization, FIND 2012; McMorrow *et al*. 2011).

2.5.5.2.2 pLDH detection RDTs

The pLDH enzyme is produced by all human malaria parasite species, and persists in the blood for a shorter period of time (seven days) than the fourteen days HRP-2 persists (so can be used to investigate treatment failures). The pLDH rapid diagnostic tests have higher sensitivity and specificity compared to both microscopy and HRP-2 rapid diagnostic test in testing for *Plasmodium falciparum*. They are more sensitive to heat and humidity, making it difficult to use in tropical and sub tropic regions (Dzakah *et al*. 2014; Wongsrichanalai *et al*. 2007; World Health Organization 2006; Warrell & Gilles 2002).

2.5.5.2.3 Aldolase (pALDO) RDTs

The most common form of Aldolase rapid diagnostic tests for malaria is the pan Aldolase RDTs that detect all plasmodia aldolase. Their relative sensitivity to nonfalciparum Plasmodia is low (Dzakah *et al*. 2014), so the current advancement in the development of Plasmodium vivax specific aldolase RDT is very welcoming.

2.5.5.3 Fluorescence Microscopy.

This depends on blood films stained with acridine orange or benzothiocarboxypurine and read on fluorescence microscopes or microscopes with fluorescence attachment.

Its execution is effortless, quicker to screen for parasites at lower lens power and results are easy to read, but requires micro centrifuge, fluorescence microscope and specialized QBC capillary tubes (Drakeley & Cook 2009; Warrell & Gilles 2002).

2.5.5.4 Malaria Serology

Since the 1960s, Serological tests have been in used to detect malaria antibodies (Drakeley & Cook 2009). Complement fixation tests, indirect fluorescent antibody tests and indirect heamaglutination assays have been described and used in epidemiological research studies to support malaria control and elimination efforts (Drakeley & Cook 2009; Warrell & Gilles 2002).

2.5.5.4 Complement Fixation Tests (CFT)

Complement Fixation tests were the earliest documented serology tests used at the turn of the twentieth century to detect malaria antibodies in patients. It was shown then to be more sensitive than microscopy in the detection of malaria infections. It then evolved over the years to other serological tests (Drakeley & Cook 2009; Warrell & Gilles 2002).

2.5.5.5 Indirect Heamaglutination Assay (IHA)

Indirect Heamaglutination assays were used in the 1960s. It works by cross-linking malaria antibodies in patient's blood with antigens linked to red blood cells (RBCs). Clumping of RBCs lead to a positive test (Drakeley & Cook 2009; Warrell & Gilles 2002).

2.5.5.6 Immunofluorescence Antibody Test (IFAT)

This is the main method for malaria sero-diagnosis especially in the 1960s. Antigens fixed on glass slides are incubated with patient serum. A secondary antibody coupled with a fluorescent compound is then added to detect bound antibodies using a fluorescence microscope. It requires a fluorescence microscope; it is subjective to the skills level of the reader. It is also labor intensive.

2.5.5.7 Enzyme-linked Immunosorbent Assay (ELISA)

Antigens, usually a sole recombinant protein are bound to micro titer plates. Blocking agents are added to patient serum to block nonspecific sites on the antibodies and then added to the micro titer plates and incubated. Bound antibodies are detected with a secondary antibody linked with an enzyme. An enzymatic substrate is then added which is converted if there is bound enzyme in the well resulting in a color change or fluorescence. The resultant color change is then read using a spectrophotometer (Drakeley & Cook 2009; Warrell & Gilles 2002).

2.5.5.8 Protein Micro-array

These are recent additions to antibody detection methods for malaria diagnosis. They are similar to ELISA. It uses microscope slide bound recombinant proteins (in Nano grams). It has a wider range of antibody detection, and is limited to specialized research units and are very expensive to run (Drakeley & Cook 2009; Warrell & Gilles 2002).

2.5.5.9 Nucleic Acid Assays (NAA)

New methods for routine PCR-based surveillance of malaria infections are now used for research and field studies, which are more sensitive than light microscopy or Rapid Diagnostic Tests in detecting submicroscopic infections, especially with rare species (Plasmodium malariae, Plasmodium ovale and Plasmodium knowlesi), mixed infections and low-density infections. It applies DNA or RNA hybridization to diagnose malaria. A known sequence of nucleic acid is synthesized and labeled with a colour-metric reagent (may be radioactive or not). This is known as a probe, used in the detection of parasite nucleic acid. The most commonly used nucleic acid assay for malaria is the nested PCR. It uses amplification methods to increase the number of copies of target parasite nucleic acid sequence, increasing test sensitivity exponentially.

The risk of sample contamination is intrinsic with the amplification process and care needs to be taken to prevent that (Mekonnen *et al*. 2014; Warrell &
Gilles 2002)

2.6. Global Malaria Burden

Precise figures on malaria incidence allow easy tracking of disease patterns, targeting of resources to areas of greatest need and accurate evaluation of the impact of malaria interventions. The World Health Organization reported in 2013 forty-one countries from which trends in malaria epidemiology was impossible to assess from reported data because of inconsistencies in the completeness, changes in diagnostic practice or health-service use. These countries accounted for 80% of cases in 2000.

Many interventions have been rolled out to combat global malaria disease burden since 1950 (World Health Organiztion & UNICEF 2003). Since 1999, renewed efforts and better tools have been rolled out on a large scale to control, eliminate and eventually eradicate malaria (Greenwood & Koram 2014; World Health Organization
2011) and has resulted in the reduction of disease burden even in Sub-Saharan Africa (O'Meara *et al*. 2010). These "better" and expensive control tools require evaluation methods which are relatively simple and easy to conduct to provide quick, reliable and precise estimates of current transmission intensity to inform and direct control programs (Drakeley *et al*. 2005). Inferences about malaria epidemiology trends need to be based on estimates of the malaria case incidence and mortality rates.

Approximately 86% of malaria deaths globally; between 1980 and 2010 were of children under 5 years of age (Liu *et al*. 2012; Murray *et al*. 2012). The estimated incidence of malaria globally has reduced by 17% since 2000 and malaria-specific mortality rates by 26%. These rates of decline are lower than internationally agreed targets for 2010 (reductions of 50%) but nonetheless; they represent a major

achievement. Current WHO estimates state that between the years 2000 and 2012, global malaria mortality and incidence rates decreased by 45% and 29% respectively. Within the same period, malaria mortality and incidence rates decreased by 49% and 31% respectively in the sub-Saharan Africa. This major achievement has been adjudged to have resulted from scale-up of vector control interventions, diagnostic testing and treatment of malaria cases with Artemisinin-based combination therapies. (World Health Organization 2013b).

The year 2010 was set to achieve universal coverage for all populations at risk of malaria using locally appropriate interventions for prevention and case management, and to reduce the malaria burden by at least 50% compared to the levels in the year 2000 by the Roll Back Malaria, a neutral global framework, formed to provide a coordinated action against malaria.

The Roll Back Malaria (RBM) updated its targets in June 2011. The new targets were to:

- reduce global malaria deaths to near zero by end-2015;
- reduce global malaria cases by 75% from 2000 levels by end-2015;
- Eliminate malaria by end-2015 in 10 new countries since 2008.

These targets were to be achieved through:

- Universal access to case management at the community, public and private health facilities with appropriate referral systems.
- Utilization of preventive measures (Roll Back Malaria 2008; Nahlen & Low-Beer 2007)
- Acceleration of the development of surveillance systems to monitor and report progress (Nahlen & Low-Beer 2007).

The World Health Assembly also set its target to reducing malaria mortality rates by 75% by 2015. The Millennium Development Goals (MDGs), decided and approved by world leaders over a decade ago had its fourth target as, " to reduce, by two thirds, the under-5 mortality rate between 1990 and 2015" and as part of its sixth target to "Have halted by 2015 and begun to reverse the incidence of malaria and other major diseases" worldwide, through working together with Governments, the United Nations, the private sector and civil society, communities, families and individuals

(United Nations 2012).

2.7. Global Progress in Malaria Control

The decade 2000-2010 marked the beginning of increased attention to malaria control and elimination efforts, after the abandonment of the Global Malaria Eradication Campaign, and has been included in major international development targets and has been recognized as a contributor to global poverty (including the United Nations' Millennium Development Goals- halting and reversing the incidence of malaria by 2015, the Abuja Declaration of 2000 - African leaders committed to halving malaria mortality by 2010). The increased international attention led to increased funding to fight malaria using effective malarial control interventions.

Effective tools are available currently for the prevention and treatment of malaria in almost every setting, with the potential to substantially reduce the morbidity and mortality from malaria. The main preventive tools for prevention are

- long-lasting insecticidal nets (LLINs),
- indoor residual spraying (IRS)
- intermittent preventive treatment for pregnant women (IPTp)
- Diagnostic and treatment for malaria case management- Artemisinin-based combination therapies (ACTs).

Since 2000, a tremendous expansion in the financing and coverage of malaria control programs has led to a wide-scale reduction in malaria incidence and mortality. Based on reported data, 59 out of 103 countries that had ongoing malaria transmission in 2000 are meeting the Millennium Development Goal (MDG) target of reversing the incidence of malaria. Of these, 52 are on track to meet Roll Back Malaria (RBM) and World Health Assembly targets of reducing malaria case incidence rates by 75% by 2015, including 8 countries in sub Saharan Africa. From 2000 to 2012 worldwide estimated malaria mortality rate decreased by 45% in all ages, and by 51% in children five years of age and below. Based on these decreases, then malaria mortality rates are expected to fall by 56% in all ages, and by 63% in children five years and below by the year 2015. Projections of estimates indicates that 3.3 million malaria deaths were prevented between 2001 and 2012, 90% of whom were children aged five years or below in sub Saharan Africa (World Health Organization 2013). The decreases in malaria deaths in children have largely led to advancement in achieving the target for MDG 4 (to reduce, by two thirds, the under-5 mortality rate between 1990 and 2015) (World Health Organization 2013b; United Nations 2012; Lynch *et al*. 2012). Between 2011 and 2012, the rate of reduction in projected malaria mortality rates decreased, partly due to reduction in funding for malaria control interventions, especially delivery and distribution of long lasting insecticide treated nets (estimation of malaria deaths in children fewer than 5 years of age in Africa utilizes insecticidetreated net (ITN) coverage as an input).

In spite of all these achievements, millions of persons at risk of malaria still do not have access to malaria control interventions, resulting in an estimated 207 million cases and 627 000 Malaria deaths in 2012 (World Health Organization 2012c).

Since 2013, following reviews and projections of progress in control efforts, new and updated malaria control policies, operational manuals, plans and initiatives have been issued, following meetings of WHO's Malaria Policy Advisory Committee (MPAC) which was formed and operationalized in 2012, to provide strategic advice and technical input to WHO on all areas of malaria control and elimination. Following MPAC recommendations, WHO made available, guidelines on a range of policy areas, including achieving universal coverage with long-lasting insecticidal nets (LLINs).

2.8. National Malaria Burden, Control Efforts and Achievements

Malaria is endemic in Ghana (World Health Organization 2011) and is attributed to be the cause of 40.2% of outpatient visits, 35.2% of admissions, 6.8% of maternal deaths and about 18.1% mortality at health facilities in Ghana. It also causes 29.5% of deaths in patients aged under 5 years old at health facilities in Ghana (Ghana Health Service 2011b; World Health Organiztion 2011)

In 2011, 48.9% of Households had at least one insecticide treated bed-net (ITN), 39% of children less than 5 years of age slept in a bed-net the night before the Multiple Indicator Cluster Survey (MICS). Also 67.1% of pregnant women received IPTp2 and 32% of them slept under an ITN the night before the survey (Ghana Statistical Service 2012). The prevalence of malaria within Greater Accra (the region in which the study district is situated) and Volta region, the region on the eastern border of the study district) is as portrayed in the table 2.1.

Greater Accra Region of Ghana has the least malaria parasite and RDT positive prevalence rates of about 4% and 10% respectively from the MICS 2011. The Volta region, which is the eastern neighbor of the region, closest to the study district, had about 4-fold risk for both parasite and RDT positive prevalence.

Table 2.1: Malaria prevalence in the Greater Accra and adjacent Volta Region, (0-5 year olds), Ghana

Region	RDT positive	Malaria Microscopy positive	hemoglobin <8.0g/dl	hemoglobin <7.0g/dl	IPTp2
Greater Accra	10.1%	4.1%	2.8%	0.5%	42.6%
Volta	32.5%	17.3%	5.7%	1.1%	48.5%
National	47.7	27.5%	7.4%	2.5%	53.3%

Ghana Multiple Indicator Cluster Survey (MICS, 2011)

Greater Accra Region of Ghana has the least malaria parasite and RDT positive prevalence rates of about 4% and 10% respectively from the MICS 2011. The Volta region, which is the eastern neighbor of the region, closest to the study district, has about four-fold risk for both parasite and RDT positive prevalence.

Malaria case fatality has also decreased from 3.7% in 2009 to 1.2% in 2011, whiles the proportion of malaria cases tested before treatment also increased from 32.7 to 40.2% nationwide (Ghana Health Service 2011b).

2.8.1. District Malaria Burden, Control and Achievements

Malaria is attributed to be the cause of 50% of outpatient (OPD) attendance in 2008 in the Dangme West District (Ghana Health Service 2011a). Transmission peaks at the end of wet seasons in southern Ghana (Afari *et al*. 1995), where the Dangme West is situated. The incidence rate of clinical malaria as measured in 1992 was highest in the less than 10 years age group. The table below summarizes the 1992 epidemiological data.

Table 2.2: Malaria Indices in the Dangme West District, 1992 (Afari *et al*. 1995)

Results		
	Dodowa	Prampram

EIR	0.01	0.06
Man Biting Rate/bites per man night	0.79	1.54
Sporozoite rate	2%	0.7%
Peak biting density	November	June
Malaria Parasite Prevalence		
April (Dry)	42.2%	19.8%
August (Wet)	51.3%	26.6%
Malaria Incidence/1000 population	106.6	68.5

The methodology used in the 1995 study covered two malaria parasite prevalence surveys covering 600 participants each timed to coincide with the end of the dry and wet seasons in two communities, the Dodowa and Prampram Townships. A cohort of 6558 from Dodowa and 6682 from Prampram was followed once every 2 weeks for a year. This was augmented with weekly human landing mosquito catches from the same communities. Dodowa had a malaria parasite prevalence of 42% and 51% for April (Dry) and August (Wet) respectively, whilst Prampram had 20% and 27% malaria parasite prevalence for the same months respectively. The Malaria Incidence per1000 population was 107 for Dodowa and 69 for Prampram.

CHAPTER 3: METHODOLOGY

3.1. Study Methods and Design

This was a three-part study, comprising of:

- Malaria parasite prevalence study, which employed an observational crosssectional design.
- Malaria incidence study, which used a prospective, cohort non-interventional study design to estimate malaria incidence.
- Entomology study, which used a cross-sectional design.

3.2. Malaria Parasite Prevalence Study

3.2.1 Sample Size Estimation and Sampling

A total sample size of 3465 (21 communities, 55 persons per community, yielding 1155 per zone) would provide 80% power to detect an 8% difference in malaria parasite prevalence at the 5% significance level, assuming a minimum prevalence of 22% (DSS unpublished data (Dodowa Health Research Center 2011) and refusal or failure to find 10% of participants from the DSS.

3.2.2 Data Collection Techniques and Tools

A cross-sectional survey covering 3500 persons of all ages and sexes of age, selected using multistage sampling from the four sub districts (corresponding to the three ecological zones) was done at the end of the Wet Season in 2011 (August). It was then repeated to coincide with the end of the dry season (March in 2012) to determine the malaria prevalence for the wet and dry seasons respectively for the Dangme West district.

Participants were interviewed using a questionnaire after a written informed consent had been sought. Consent was sought after the reasons for the study and its procedures had been

thoroughly explained to the participants. The questionnaire covered demographic information, sex, age, household number, community of residence, use of bed nets, history of fever in the last 2 weeks and actions taken if any. The weight and height of participants were taken to guide antimalarial dosing if needed. Axillary temperatures of participants were taken using an electronic thermometer. A finger prick/ heel prick/venous sample was taken for RDT on the spot and the rest of sample taken to the Laboratory at Dodowa for thin and thick films, hemoglobin and filter paper blots.

Participants found to be RDT positive with or without fever were given free antimalarial according to national antimalarial dosing guidelines. Those with severe malaria or any other ailment were given appropriate initial treatment and referred to the nearby health facility for treatment.

Participants who were pregnant with gestational age 32 weeks or more, irrespective of parity and maternal age or had delivered 6 weeks or less were interviewed with a questionnaire which sought the use of malaria interventions targeted at pregnant women. After written informed consent was sought, information on age, name, obstetric data, use of antenatal services, history of any ill health since the last two weeks, IPTp uptake, antimalarial and bed net use was sought. A finger prick/venous blood sample was taken for RDT, malaria parasite identification and hemoglobin determination. All pregnant women who were RDT positive, or had fever, or defaulted on IPTp or had any other ailment were referred to appropriate health facility.

3.2.3 Study Variables

- Age
- Sex

- Zone of Residence
- Fever prevalence
- Malaria parasite prevalence
- ITN use
- ITN ownership

3.2.4 Study Endpoints

1. Malaria parasite parasitaemia/ RDT positivity (for prevalence study)

3.2.5 Study outcomes

Malaria prevalence by age and zone, RDT positive prevalence by age and zone. Malaria relative risks by age and zone of residence were also generated.

3.3. Cohort (Incidence) Study

3.3.1 Sample Size Estimation and Sampling

A sample size of 2145 (13 communities, 55 persons per community, 715 per zone) provided an 80% power to detect a difference of 0.10 in incidence rate between the 3 zones at a significance level of 5%, assuming a minimum prevalence of malaria of 22%. An allowance of 25% loss to follow up and 10% refusal (and failure to identify participants drawn from the DSS) was then added. Final sample size was 2896. 260 newborn babies were enrolled on a rolling basis to the cohort. The final sample size was 3156.

Source: Hayes and Bennett, Simple Sample size calculation for cluster-randomized trials, IJE (1999) equation.

3.3.2 Sampling

Multistage cluster sampling was used to select communities. The list of communities in each sub-district was generated at Dodowa using the DSS.

In each zone, 13 communities were selected by simple random sampling for the cohort study. After selection, the population of the community was checked. Communities with population less than 100 members were then added to the nearest community or two to form a cluster with at least 100 people. Within each cluster, 75 persons were then selected by random sampling from the cluster list of residents generated from the DSS.

The cohort study was undertaken to determine malaria incidence for a period of one year. It was done by selecting and following a cohort of persons of all ages from the district. The cohort of 2896 participants of all ages, 966 from each ecological zone were selected using multistage cluster sampling and followed once a month for a year by trained field workers. At each visit, a history of fever within the past 2 weeks was elicited. Those who responded yes were interviewed with a questionnaire, which sought what actions had been taken. ACT and ITN use was verified, after which a finger/ heel prick blood sample was taken for RDT on the spot, and the rest of the sample sent to the Lab at Dodowa for thick and thin blood film preparation.

Those who are found to be positive and had not had any treatment were then treated with anti-malarial. Those who were negative but had axillary temperature above 37.5°C were referred to the nearest health facility.

For the cohort study, clinical malaria was defined as a history of fever in the past 2weeks plus positive RDT test, or temperature ≥ .37.5°C with positive peripheral parasitaemia by microscopy.

3.3.3 Study Population

All persons residing within the Dangme West District at the time of study

3.3.4 Study Variables

- Age
- Sex
- Community/ zone of Residence
- ITN use
- Clinical Malaria
- History of fever in the last to weeks
- Antimalarial drug use in the last two weeks
- Malaria incidence rates
- ITN ownership

3.3.5 Study Endpoint

2. Clinical malaria (history of fever in the last 2 weeks and positive RDT for cohort study)

3.3.6 Study outcomes

Malaria incidence rates by age and zone, Relative incidence rates by age and zone.

3.4. Entomology Study

An entomological study to assess malaria transmission intensity in the Dangme West district using entomological inoculation rate (EIR) was undertaken.

Using the DSS, 4 houses per zone were randomly selected for mosquito collection per month for 12 months (April 2011 to March 2012). Collections were done using Human Landing Catches (HLCs) and Pyrethrum spray catches (PSC).

3.4.1 Human landing collections (HLC)

Four trained and consented fieldworkers with a supervisor collected the mosquitoes. At each selected house, the supervisor went ahead to seek informed written consent from the head of household before collections started. Two collectors sat indoors and two outdoors for 50 minutes of each of the hours between 1800hrs and 0600hrs to aspirate mosquitoes, which landed on the exposed legs with the help of flashlights and glass tubes. In order to compensate for differences in individual attractiveness to mosquitoes and as a precaution against dozing and inappropriate techniques, the two teams of collectors rotated between indoors and outdoors hourly after taking ten minutes break. In addition, the supervisor made surprise visits throughout the night for quality assurance and to gather the mosquitoes collected.

Field workers were tested each month for malaria and were given free and rapid treatment when they showed suspected clinical signs of malaria according to national malaria control guidelines for treatment of malaria and on the basis of fever and detectable *P. falciparum* parasitaemia. They were also given prophylactic ivermectin and albendazole against lymphatic filariasis according to national guidelines on filariasis prophylaxis.

The captured mosquitoes were placed into paper cups covered with nets to prevent the mosquitoes from escaping and labelled according to the location, date of collection, site of collection (indoors or outdoors), collector's name and hour of collection.

The collected mosquitoes were placed upright in specially made wooden boxes and transported to the laboratory at Dodowa. Pieces of wet cotton wool were placed over the tops of the cups to maintain the mosquitoes at an appropriate humidity.

3.4.2 Outdoor Collections

During the day (between 0900-1100 hrs.), the team searched for outdoor shelters for resting adult anopheles mosquitoes, within the compound of the house where collection was done the night before. These were aspirated into paper cups, labeled and sent to the laboratory at Dodowa.

3.5 Laboratory Procedures

All blood samples for both prevalence and cohort studies were collected into 1ml vacutainer EDTA tubes and transported in ice bins to the laboratory at Dodowa for processing. At the lab, the samples were logged and stored in a blood fridge and processed within 3 days.

3.5.1. Processing of Blood Smears for prevalence and cohort studies

Thick and thin blood smears were prepared and stained with 3% Giemsa for 45-60 minutes, washed and then dried by experienced Technicians at the Dodowa hospital laboratory. Slides were then examined under oil immersion with a light microscope under a magnification of 100. The thin part of the slide was for parasite identification whiles the thick part was used for malaria parasite quantification by 2 experienced microscopists. Blood asexual parasites were counted against 200 white blood cells and sexual parasites were also read against 200 white blood cells. A blood slide was declared negative only after reading 200 high power fields. Parasite densities were estimated by counting the number of parasites per 500 white cells in a thick film. The parasite counts were then converted to parasite counts per micro liter of blood (µl) by

multiplying the number of parasites by 8000 and dividing the answer by the white blood cell count (World Health Organization 2010a). Where 2 microscopists disagreed on the presence (positive smear) or absence (negative) of asexual stage parasites in a thick smear, a third microscopist read the slide and his reading was final. All dried blood smears were packed in slide boxes, each containing 100 slides, labeled and stored in the laboratory. All preparation and readings were done under the supervision of the Senior Laboratory Technologist who had been trained and was already working on other malaria studies at the site. Positive blood film for malaria or malaria parasitaemia was defined as the presence of asexual blood stage parasite of any Plasmodium species detectable on a thick blood smear.

3.5.2 RDT Processing

All RDTs from the prevalence study were done according to the manufacturer's instructions (First Response). After reading and recording the results in the field, they were dried for about 2 hours and packed with desiccant (silica) into sample bags and packed in bins and transported to the laboratory at Dodowa in with ice packs. At Dodowa, they were kept in a fridge at a temperature of 4°C until selected and sent to Noguchi Memorial Research Institute for Medical Research for processing (serology).

3.5.3. Hemoglobin Measurement

Blood collected by venous (adults) or capillary children were used to determine the hemoglobin level at the laboratory at Dodowa using an automated Haemo-analyzer for all participants of both prevalence studies. Samples collected were run the next day and results and referrals of those with anemia communicated to them by mobile phones (all participants gave phone numbers by which they can be contacted).

Anemia was defined as blood Hemoglobin level less than 11g/dl. Mild anemia was defined as blood hemoglobin level < 11.0g/dl >8.0g/dl≤. Moderate Anemia was defined as blood hemoglobin level <8g/dl ≥5g/dl, and Severe anemia as blood hemoglobin level <5g/dl. Malarial anemia was defined as a blood hemoglobin level <8g/dl plus positive peripheral blood malaria parasitaemia.

3.5.4 Filter Paper Blots

Filter paper blots were prepared for all prevalence study participants at the lab in Dodowa. They were air dried at room temperature, packed into Ziploc bags with desiccant and stored below minus 20°C, until it was transported to NMIMR for processing.

3.5.5 ELISA Procedure

Approximately 10% percent of the total number of RDT cassettes were selected randomly (by age grouping sex and zone) per season (737 in the dry season and 761 in the wet season) and transported to Noguchi Memorial Institute for Medical Research. RDT cassettes were disassembled and blood stained parts processed to elute sera for IgG recognizing Plasmodium falciparum antigen, merozoites specific protein1$_{19}$ (MSP-1$_{19}$) using indirect Enzyme Linked Immunoabsorbent Assay (ELISA) as described by Drakely *et al* (Drakeley *et al*. 2005).

Nine-six (96) well ELISA plates (Falcon Cat no: 3910 BD) were coated at 100ul/well with MSP1 FVO at 1ug/ml and incubated overnight at 4^{o}C. Columns 1 and 2 were coated with pure IgG (starting at 200ng/ml with a 2 fold serial dilution) to serve as a standard curve. Plates were allowed to warm to room temperature and washed 4X with (0.005% tween20 in PBS). Blocking was done with (5%milk in PBS) at 200ul/well and incubated for 1hour in a humidified chamber. Plates were washed 4X with

PBS/tween. The eluted samples were diluted **20X** and added at 100ul/well in duplicates from column 3 to column 12. (Blocking buffer was added to columns 1 and 2 as these were coated with known concentration of pure IgG, titrated 2 fold, to serve as a standard curve). Plates were incubated overnight at 4^{o}C. Plates were recovered and allowed to warm to room temperature and washed 4X with PBS/tween. Rabbit anti-human IgG/HRP (Horseradish Peroxidase) conjugate was diluted at **1:2000** and added at 100ul/well and incubated for 1hour in a humidified chamber. The plates were washed 4X with PBS/tween. Plates were then developed with 100ul/well of TMB substrate for 20 minutes. The reaction was stopped with 0.2M H_2SO_4 and Optical Densities read at a wavelength of 450nm. Optical Densities (OD) were converted to concentrations using the Auditable Data Analysis and Management System for ELISA (ADAMSEL) spreadsheet by EdRemarque. Initial 10% of filter paper blots were processed to elute sera for IgG recognizing Plasmodium falciparum antigens using indirect ELISA. The filter blots eluates assays were then compared with RDT assays using filter blots as Gold Standard.

3.5.6 Processing of mosquitoes

Mosquitoes captured from the HLCs were killed with chloroform and the anopheles sorted out from the other genera and identified morphologically into species (Gilles & DeMeillion, 1968) and counted. Each anopheles was put into an Eppendorf snap vial and the vials placed into zip-lock plastic bags with pockets of desiccant according and labeled according to date, area, and hour of collection, collector and species and stored. All the mosquitoes in the vials were then removed and checked for sporozoites (infectivity). The heads and thoraces of all collected mosquitoes were checked for presence of circumsporozoite antigens (CS) of *P. falciparum, P. malariae* and *P. ovale.* This involved using monoclonal antibodies specific for the major surface coat proteins

of these parasites in an enzyme-linked Immunoabsorbent assay (ELISA) as described by Beier et.al. 1990). The abdomen of blood engorged anopheles mosquitoes were analyzed by indirect ELISA using polyclonal rabbit antisera to identify the host blood meal as to whether they are human, bovine, ovine, caprine, equine or avian host (Service, 1986). The sporozoite rate was estimated as the proportion of mosquitoes, which were ELISA positive for CS protein. The entomological inoculation rates (EIR) were calculated as the product of mosquitoes landing on a volunteer per night for a given place (Man Biting Rate, MBR) and the sporozoite rate from that time and place. The human blood index (HBI) is the proportion of female anopheles that had fed on human blood.

3.6 Staff Recruitment, Training and Management

Field workers, health staff, supervisors and research assistants were recruited in consultation with project managers at the research Centre. Some workers who had excelled from some phasing out projects were recruited after undergoing interviews at Dodowa.

The teams were as follows:

Entomology Study

- 5 field workers, 1 lab technician all full time

Cohort Study

- 5 field workers, 1 supervisor all full time

Malaria Prevalence Study

- 1 Doctor, 1 Clinical Nurse, 2 Community health Nurses, 4 lab technicians and 6 field workers (divided into 2 teams for surveys, hired for 1 month for each survey)

Back office

- 1 Research Assistant
- 3 Data entry clerks
- 2 Laboratory technologists (part time)
- 6 Microscopists (part time)

3.6.1 Training

Prior to the onset of training, the transport officer of the Greater Accra Office of Ghana Health Service did motorbike training for all field workers, supervisors and research assistant for 2 weeks. This covered good riding techniques, maintenance of bikes and safety on the road. Those that did not have valid riding licenses then applied for and obtained one after the training.

The entomology team then travelled to Navrongo Health Research Centre for a twoweek training session where they were trained on record keeping, methods of mosquito collection, safety and comportment at the community level. Field workers were also trained and supervised to ensure confidentiality and privacy of room owner's volunteered rooms for mosquito collections. The lab technician was trained for a week on sorting, storing, documenting and dissection of mosquitoes.

The prevalence study team was trained for 5 days on community entry, obtaining individual consent, questionnaire administration, importance of standard operating procedures, documentation, taking of Axillary temperature, taking finger prick blood/venous samples, transporting of blood samples, antimalarial dosing, infection prevention, anthropometric measurements and performing Rapid diagnostic tests for malaria.

Malaria Incidence study team was trained for 10 days on community entry, mobilization, obtaining community and individual consent, questionnaire administration, importance of standard operating procedures, documentation, taking of axillary temperature, taking finger prick blood samples, transporting of blood samples, antimalarial dosing, and infection prevention and performing Rapid diagnostic tests for malaria. Data entry clerks were trained for a week on data entry and management.

Microscopists and Laboratory technologists at the Dodowa Hospital had already been trained at Kintampo Health Research Centre and were working on malaria projects in the district already. They were then oriented on the standard operating procedures of the study for a day.

There were monthly project meetings where all staff working on various part of the project met. Issues arising were resolved, progress was reported and feedback given. The principal investigator chaired these meetings.

Protective clothing was provided for all field workers. Use of appropriate clothing was supervised to ensure staff adhered to safety standards whiles spraying or doing field work. This included head, overalls, gloves, goggles, raincoats, respirators, torchlights and wellington boots.

3.7 Pre-testing

Questionnaires and all study tools were tested using role-play during training on volunteers. Field pre-testing of data collection tools was done at Bawaleshie and Mokomeshitamohe, two communities close to Dodowa (which were not part of the study) before study initiation.

3.8 Data Handling

Completed questionnaires and forms were checked for errors and consistency. The completed questionnaires were then batched, stored in locked bins, and removed for data entry in batches. Data were doubly entered in EPI Data and cleaned and databases generated in Microsoft Excel for further cleaning.

3.9 Data Analysis

The database was exported into STATA11 for analysis.

Clustering was adjusted for by using the "svy" command in STATA. Adjusted frequencies and proportions were generated. The study samples were categorized into three zones (main exposure) according to community of residence, with three levels, Dodowa (Forest), Prampram (Coastal) and Osudoku (Lakeside).

Confounding was dealt with by adding the confounding variables to the regression model (sex) or by stratification on the confounding variable (age) and estimating the parameter within each stratum. Weighted averages of the stratum-specific rate ratios were calculated using the Mantel–Haenszel approach in STATA, The weight given to each stratum was determined by the precision with which the corresponding stratumspecific Rate Ratio was estimated. Both age and sex were also dealt with by including them in the regression model. Age was categorized into five groups, zero to ten years, eleven to twenty, twenty-one to thirty, thirty-one to forty and forty-one years and above.

The risk of disease in a population is a measure of disease frequency and is commonly interpreted as prevalence. It is represented as:

D (# disease)/ N (population)

Risk ratio of a disease (or relative risk) is a measure of relative effect of a disease and measures the strength of association. It is represented by:

Risk in exposed / Risk in unexposed

Crude risks and rates were derived. Logistic regression analysis was then used to model relationship between quantitative outcomes and exposures of interest whiles adjusting for confounding. Significance tests were used to test strength of associations and regression diagnostics used to check model assumptions.

Hazard ratios based on time of follow up were generated for the incidence data. The proportional hazards assumption is the fundamental assumption of the Cox regression model. The rate (or hazard) ratio of a subject i relative to a subject with zero values of all the explanatory variables, $\theta i = \lambda (t:i) / \lambda(t:0)$ is constant over the entire followup time, that is, the all-time variation in the individual rate $\lambda(t:i)$ is captured by the baseline rate $\lambda(t:0)$.

3.10 Ethical Consideration

Ethical approval was sought and obtained from KNUST (CHRPE 189/10) and Ghana Health Service (GHS-ER: 03/5/11).

3.10.1 Community Mobilization and Consent Seeking

Several Meetings were held with the District Director of Health Services and Director of the Research Centre right from the problem identification stage to proposal development and ethical approval. Letters were also written to the directors to seek approval for the study to be conducted in the District.

Once the communities were selected, letters explaining the objectives, procedures, benefits and other consequences including the public health impact of the study were

written to the leaders of all the selected communities, which were then followed up with visits and meetings with opinion leaders within the communities.

Community meetings were conducted to mobilize participants for the study. Invitations to meeting and surveys were done using announcements in the local dialects using megaphone-mounted pickups with community health nurses as well as town "criers" in the communities during the study period.

3.10.2 Participant Enrolment

Within each community, after seeking community consent, selected houses and participants for each study were identified. House and individual identities, which could not be verified were noted and brought to Dodowa for replacements for the cohort and entomology studies before the study started.

3.10.3 Cohort (incidence) Study

All persons living residing within the Dangme West District within the study period, intending to live within the district for the next 12 months and consenting after aims, objectives, risks and benefits of study have been explained to them qualified to be enrolled into the study. Once individuals were identified, Written Informed Consent was sought from participants and caregivers (in the case of children) after the objectives, the risks and benefits of participating in the study had been explained to each participant or caregiver. In older children (11- under 18 years), in addition to the written consent from caregivers, assent was also sought from them.

Inclusion Criteria:

All persons living residing within the Dangme West District who consented to participate in the study after aims, objectives, risks and benefits of study had been explained to them.

The Exclusion criteria included the following:

- Non-consenting to participate
- Not resident in the Dangme West district
- Not intending to live in the district for the next 12 months for follow up
- Presence of acute or severe disease at the time of enrolment

3.10.4 Malaria Prevalence Study

All persons in the Dangme West District who had resided at least for 6 months prior to the day of the study and consenting after aims, objectives, risks and benefits of study had been explained to them qualified to be enrolled into the study.

Community leaders were informed prior to the day of the community survey with written letters and visit by a field supervisor. Gong beating or megaphone announcements on the survey dates time and place of meeting were done a day or 2 before the survey.

On the day of survey, field supervisors and workers went ahead of the survey team, identifying and mobilizing participants to an identified survey point within the community.

Once individuals were identified, Written Informed Consent was sought from participants and caregivers (in the case of children) after the objectives, the risks and benefits of participating in the study had been explained to each participant or caregiver.

In older children (11- under 18 years), in addition to the written consent from caregivers, assent was also sought from them.

Participants who could not be identified were replaced from the list of possible replacements, which had been generated from DSS.

Inclusion Criteria:

All persons living residing within the Dangme West District who consented to participate in the study after aims, objectives, risks and benefits of study had been explained to them.

The Exclusion criteria included the following:

- Non-consenting to participate
- Not resident in the Dangme West district

Presence of acute or severe disease at the time of survey

3.11 Strengths and Limitations of Study

Selection Bias: - We completed 79% of total anticipated visits which just falls short of the much accepted 80% (Sackett 1979) in the cohort study (28543 out of 36941). Most of the absences were due to travel outside the district mainly for work and other purposes. Analysis of non-responders, especially for the prevalence study showed that they did not differ much from responders in terms of age, zone of residence and sex. Selection bias is prevented if all the members of the population are given an equal chance of being selected and partaking in the study. The use of DSS to select participants gave every community in the district an equal chance of being selected.
The use of cluster sampling meant that members of both smaller communities (by population) and bigger populations had equal probability of being selected and participating.

The sample size of over 3398 and 3456 for the prevalence studies was larger than similar studies,(Starzengruber *et al*. 2014; Drakeley *et al*. 2005) and compares with the 2011 MICS which covered 4511 participants nationwide in Ghana (Ghana Statistical Service 2012). The final sample size had 80% power to detect an absolute difference of 5% or more in prevalence, and for incidence. The observation of no significant

difference for both prevalence surveys for the zones meant there was unlikely to have been an important difference to detect. Both sample sizes were calculated assuming a 15% loss to follow up and 10% inability to identify participants selected using the DSS.

For the prevalence study, the time of community visit led to the exclusion of some selected participants who were then replaced by those present in the community at the time of Visit using the DSS list for that cluster. Visits during working times meant some workers who worked outside the community at the time of visit were excluded. Pampram had no meteorological substation, so climatic data from Ada; a nearby coastal town with a substation was used as proxy.

Observer Bias: - Weighing scales and digital thermometers used for fieldwork were standardized at the Ghana Standards Board. Weighing scales were checked against standard weights each morning before use in the field.

Microscopy Results: - trained microscopists at the Navrongo Health Research Centre double read Blood Slides. There was blinding of blood slide reading, so microscopists did not know from which area the slides came. A third reader whose result was entered as the final result then read slides with discordant results. The agreement between the initial readers was 86% for the dry season, and 90% for the wet season. This is good agreement, compared with the expected agreement of 78%.

Recall Bias: - Questions were asked uniformly between participants. Emphasis on questions did not differ from zone to zone or from participant to participant. Also, neither fever (with a prevalence of 19% in children under 5 years (Ghana Statistical Service 2012) nor malaria are rare occurrences in the communities; both are also serious enough for participants to remember, so that such an event is not likely to have been forgotten. The ability to recall such an event within a two-week period was not

difficult for participants to do for both prevalence and cohort studies. The use of drug charts, used drug envelops, road to health charts and Antenatal cards helped to verify information given. Though, the mix of loss to follow up, response recall and observer bias may combine to underestimate malaria incidence reported in this study.

Generalizability: - Considering the population profile of the district, and the fact that it has good representation of both rural and urban areas, the participants could be said to be representative of any district in southern Ghana. The inclusion criteria were flexible enough which enabled many participants to partake giving the results external validity.

Internal validity: - A study has internal validity when the process of selection of study participants is carried out well, such that errors in measurement are minimal, and any observed effect can thus be attributed to the exposure being investigated. This study has internal validity because it was well designed and the selection of participants carried out properly.

Conclusion: - The main strength of this study is that it was well designed, implemented and had both internal and external validity. The sample size was large enough to answer the research questions and enough measures put in place to minimize potential biases, which could have affected the integrity of results

3.12 Assumptions

The proportional hazards assumption, which underlies the Cox regression analysis, implies that the effect of the parameters included in the model do not change during the time covered by the study. In reality, most parameters change (for example, age), but the assumption is that the change is not as large enough to the affect the true value of outcomes at the population level largely due to the short period of follow up (one year).

3.13 Study Flow Diagrams

Figure 3.1 Flow Chart showing participant recruitment and inclusion in the Prevalence Study

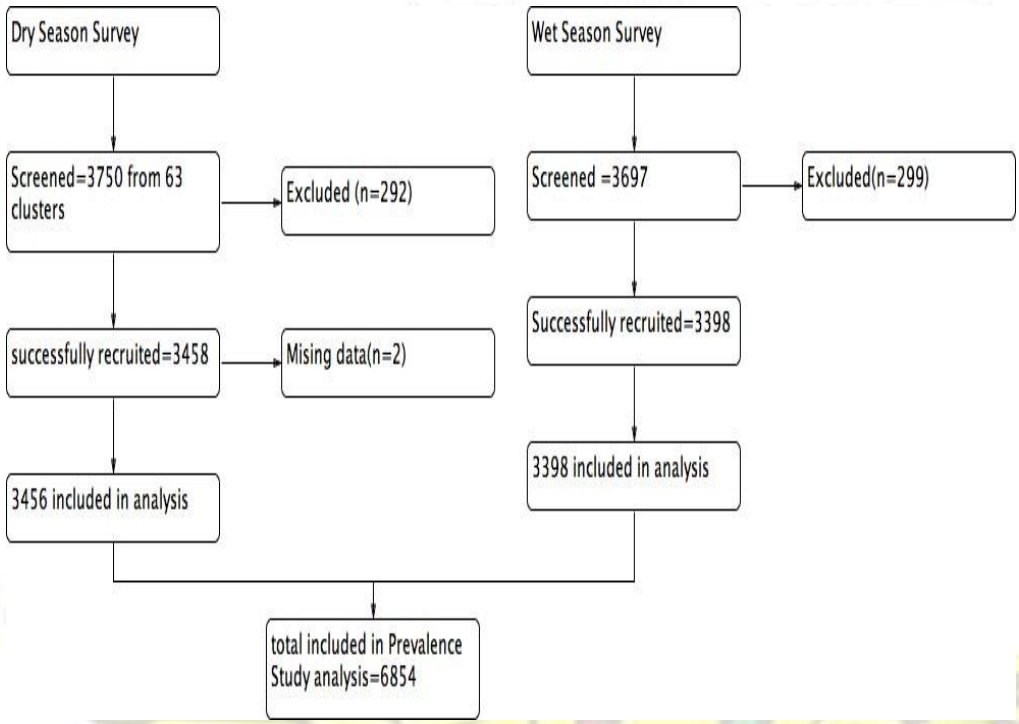
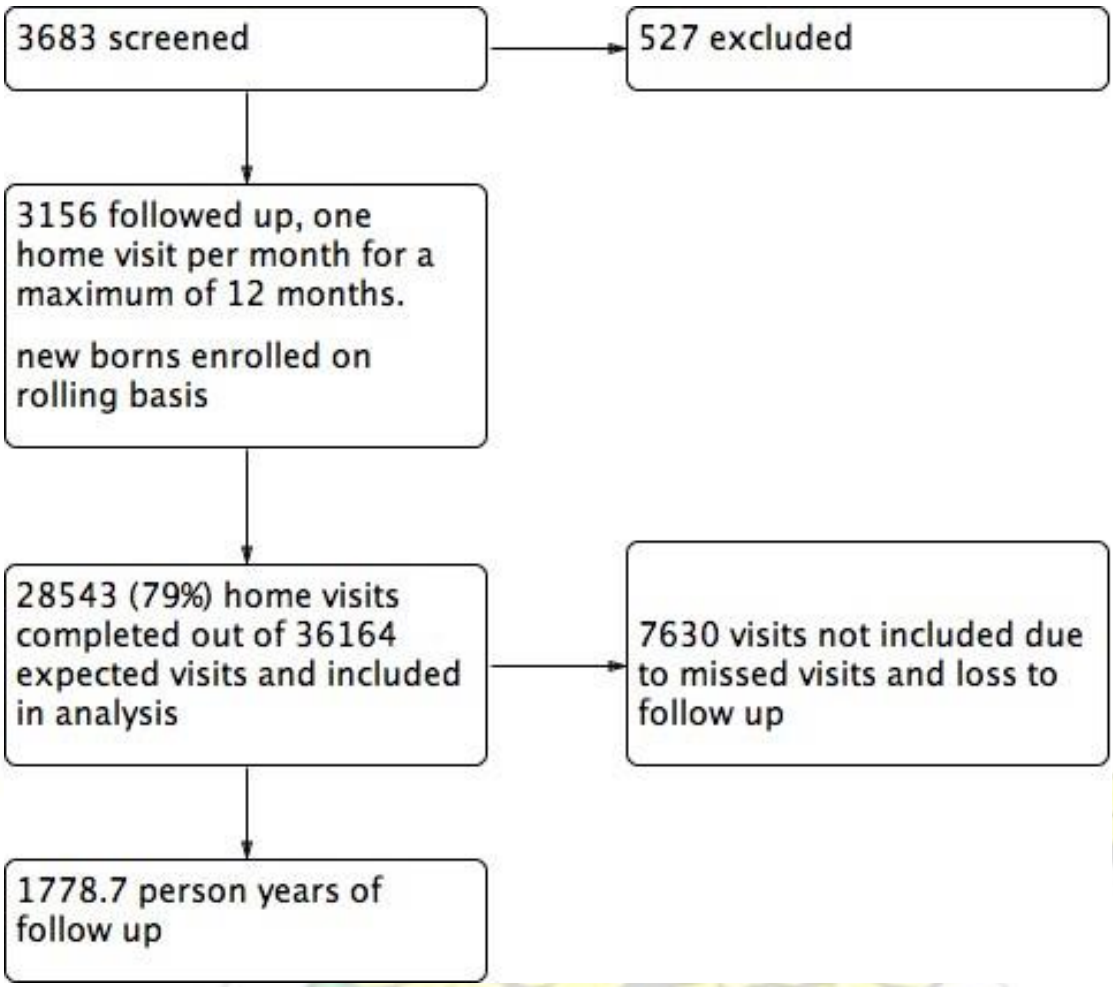


Figure 3.2 Malaria Incidence study flow chart



CHAPTER 4 RESULTS

4.1: Malaria Prevalence Study

The results of the two repeated prevalence surveys are presented in Table 4.1. The wet season survey was the first to be conducted. RDT and malaria parasite (microscopy) prevalence as well as fever prevalence are all weighted.

Table 4.1: General Study Description by season; in frequencies and proportions.

Period of data collection	March 2012	August 2011
# Clusters	63	63
#Participants Screened	3750	3697
#Participants included	3456	3398
#Participants excluded, (inadequate blood sample, severe disease, missing questionnaire or labels)	292	299
# Participants with missing Data	2(0.06%)	0
% RDT positive	17.1	24.3
% Parasite positive (by microscopy)	7.1	6.8
% Temperature ≥37.5°C (clinical fever)	0.7	2.3

Clinical fever, Parasite and RDT positive prevalence are weighted. Dry Season participants n=3750 and Wet Season n=3398 drawn from 63 clusters for each season. There was no significant difference between malaria parasite prevalence between the wet and dry season, staying at about 7%.

The wet season fever prevalence was over 3 times the dry season prevalence. The RDT positive prevalence was over 2 times the parasite prevalence in the dry season (17:7), but about 3 times the parasite rate in the wet season (24:7) (Table 4.1).

Weighted Parasite prevalence for dry and wet season was the same.
The microscopy versus RDT results are presented in Table 4.2 and are consistent with the 2011 Ghana Malaria Indicator Cluster Survey (MICS) results for Greater Accra Region in the dry season (4.1% parasite positive prevalence versus 9.8% RDT positive prevalence).

Table 4.2 shows Blood Slide versus RDT results, by season. All prevalence values are not weighted.

Table 4.2: Microscopy versus RDT Results for Dry and Wet Season Prevalence

Dry Season				Wet Season			
	Blood Smear Results				Blood Smear Results		
RDT Results	**Positive**	**Negative**	**Total**	**RDT Results**	**Positive**	**Negative**	**Total**
Positive	172	423	595	Positive	161	764	925
Negative	53	2808	2861	Negative	55	2418	2473
Total	225	3231	3456	Total	216	3182	3398
Sensitivity	0.76			Sensitivity	0.75		
Specificity	0.87			Specificity	0.76		
Positive value	0.29			Positive value	0.17		**predictive** predictive
Agreement	86.23%			Agreement	86.23%		
Expected agreement	78.51% agreement			Expected	78.51%		
Kappa	0.389			Kappa	0.389		
***p*-value**	<0.0001			*p*-value	<0.0001		

Sensitivity, specificity, predictive values and Kappa values for both seasons with their p-values are presented. Dry Season n=3456, Wet Season n=3398, N=6854. Sensitivity of RDT using microscopy as the gold standard was about 85% for both seasons whilst specificity was higher in the dry season.

The agreements levels between RDT and microscopy results for both seasons were higher than the expected levels of about 78.5% for both seasons.

4.1.1 Dry Season Prevalence

The general characteristics of participants in the dry season malaria prevalence survey are presented in Table 4.3. The Dodowa zone had the lowest number of participants and percentage of males participating in the dry season survey. The Osudoku zone had the highest Insecticide treated nets (ITN) ownership, but the difference in ITN ownership between the zones was not statistically significant. It also had the lowest parasite prevalence of 5.6%, against a district-weighted average of 7.1% and the lowest

RDT positive prevalence rate of 16%. Dodowa and Prampram had almost the same RDT and parasite positive prevalence rates with Dodowa (19.1 and 7.3%) leaning on the higher side in RDT positive prevalence while Prampram (18.5% and 7.8%) leads slightly in the parasite positive prevalence respectively.

Table 4.3: General Descriptive Summary Dry Season with weighted Prevalence (n=3750) by zone

Variable	Dodowa (Forest)	Osudoku (Lakeside)	Prampram (Coastal)	Total	*p*value
Communities Sa	21	21	21	63	
#Participants screened	1250	1250	1250	3750	
# Participants enrolled	1097	1142	1215	3454	
% Male	35.5	41.2	41	39.7	0.05
%ITN ownership (95% CI)	32.7 (29.1-36.5)	36 (33.6-38.5)	32.4 (29.3-35.5)	(31.6-35.5)	0.19
% RDT positive (95% CI)	19.1 (16.1-22.4)	16.2 (14.6-18.8)	18.5 (15.8-21.6)	18 (16.3-19.7)	0.28
%temperature ≥37.5°C (95%CI)	0.34 (0.1-0.9)	1.01 (0.6-1.7)	0.75 (0.4-1.4)	0.73 (0.5-1.1)	0.2
%Malaria Parasite Positive (95% CI)	7.3 (5.7-9.2)	5.6 (4.2-7.8)	7.8 (5.8-10.4)	7.1 (5.8-8.5)	0.28
%MSP-1_{19} positive (n=737)	47.6	46.8	46.3	46.8	0.96

Frequencies and proportions with p-values are presented. Dodowa n=1250, Osudoku n=1250, Prampram n=12750. 296 were not included in the prevalence analysis because they did qualify for enrollment. Osudoku with the highest ITN ownership had the lowest malaria parasite prevalence of 5.6% compared to Dodowa and Prampram with 7.3 and 7.8% prevalence respectively.

Osudoku zone had the lowest prevalence of RDT positivity (16%) and malaria parasite prevalence rate (5.6%) but had the highest measured fever prevalence of 1% on the day of study; whiles Dodowa zone had the lowest measured fever prevalence of 0.34%. the District average fever prevalence was 0.73%. Crude MSP-1_{19} positive prevalence did not vary by zone. Males formed about 40% of the sample in general; Dodowa had the least male representation of 35%. Insecticide Treated bed-net (ITN) ownership was

highest with Osudoku (36%) whiles both Dodowa and Prampram had 33% and 32% respectively.

Table 4.4: Characteristics of the Dry Season Survey by age group for the whole district (all zones). n=3454

	0-10	11-20	21 -30	31-40	41+	Total	*p*-value
Total participants, N	1253	521	497	334	849	3454	
%N	37.9	14	14.7	8.7	24.7	100	
% Male	41.4	34.8	41.6	36.2	40.2	39.8	0.51
%RDT positive	17.6	18.8	18.7	16.4	18.2	17.9	0.95
%Parasite positive (by microscopy)	6.7	9.4	6.6	6.3	6.9	7.1	0.59
% MSP-1_{19} positive	50.6	48.9	55.1	53	60.5	53.2	0.51
%ITN use night before survey	28.2	24.6	31.1	25	27.6	27.7	0.47
%temperature≥37.5°C	0.3	2	0.9	0.4	0.7	0.7	0.28
%Hemoglobin < 8 ≥ 5 g/dl (moderate anemia)	5.4	7.8	4.7	2.9	5.6	5.1	0.25

Frequencies and weighted prevalence are presented with p-values. The 11-20 year age group had the highest malaria parasite prevalence of 9.4% with the 31-40 year age group having the least prevalence of 6.3%.

Though all age groups were represented, the 0-10 age group was oversampled representing about 38% of the total participants surveyed (Table 7). The age distribution also reflects the mode of data collection and was disproportionately skewed towards the zero to nine years age group. In the dry season, the 0-10 years formed almost 38% whiles 11-20 formed 14%, 21-30 about 15%, 31-40 almost 9% and above 40 years almost 25%. Male representation was the least in the 11-20 years old age group with about 35% with the highest of about 42% in the 21-30 years old age group. RDT positive prevalence did not vary by age group. The 21-30 year group had the highest reported ITN use the night before survey and the 11-20 year group had the

highest moderate anemia and malaria parasite positive rate of 7.8% and 9.4% respectively (Table 4.4).

4.1.2. Wet Season Prevalence

The wet season followed for most part the dry season patterns. Positive response to ITN ownership was lower, 25% compared with 31% for the dry season but difference was not statistically significant. RDT positive prevalence differed significantly by zone; Dodowa zone had the highest risk (37.2%) with Osudoku zone having less than half the risk as Dodowa zone (13.9%). Measured fever prevalence on the day of study was more than double the dry season risk (2.3% Wet season to 0.73% Dry season) throughout the district. In the Wet season, Dodowa zone had the highest risk of 2.9% fever prevalence (Table 4.5). Parasite prevalence by microscopy also varied significantly between Dodowa and Prampram zones (7%, 7.7%) and Osudoku zone with about half the risk (3.4%). The average district MSP-1_{19} prevalence was higher in the wet season (58.5%) than in the Dry season (53.2%) though the difference was not significant. There was also no significant variation in the zonal prevalence in the wet season.

The characteristics of participants in the wet season are presented in Table 4.5.

Table 4.5: Description of Participants in the Wet Season n=3398

Variable	Dodowa (Forest)	Osudoku (Lakeside)	Prampram (Coastal)	Total	*p*value
#Communities Sampled	21	21	21	63	
#Participants included	1159	1035	1204	3398	
% Male	35.8	41.3	35	36.3	
%ITN ownership	24.5	31.1	24.3	25.6	0.29
%RDT positive	37.2	13.9	23.6	24.4	0.0001
% Temperature≥37.5°C	2.9	2.5	2.1	2.3	0.54
%Malaria Parasite positive (microscopy)	7	3.4	7.7	6.8	0.01

%MSP-119 (n=761)	**positive**	55.1	56.2	60.4	58.5	not sig

Frequencies and weighted prevalence with p-values are presented by zone. The average ITN ownership was 26%, with Osudoku having about half the malaria parasite prevalence and RDT positive prevalence as Dodowa and Prampram.

The age group characteristics did not differ much from the dry season, and is represented in Table 4.6

Table 4.6: Characteristics of the Wet Season survey Participants by age group for the whole district (all zones)

Age group (years)	0-10	11-20	21 -30	31-40	41+	Total	*p*-value
# Participated, N	1160	566	477	328	813	3398	
n, =%N	34.1	14.9	14.6	11.5	24.9	1	
% Male	50.1	39.2	25.4	22	28.7	36.3	1E-06
%RDT positive	33.5	38	16.1	14.2	13.2	24.4	0.00001
%Malaria Parasite positive (microscopy)	9.6	11.4	3.4	3.7	3.6	6.8	0.002
%MSP-1_{19} positive (n=761)	45.7	61.6	64.4	70.1	72.1		
%ITN use night before survey	25.6	16.4	23.9	24.8	18	22	0.03
% Temperature ≥37.5°C	4.5	1.9	1.5	1.9	0.2	2.3	0.00001

n=3398 for all prevalence except MSP-1_{19}, for which n=761. Frequencies and weighted prevalence are presented with p-values. Again the 11-20 year age group significantly had the highest prevalence of both malaria parasite of 11.4% and RDT positive of 38% with the 20-30 year age group having the least malaria parasite prevalence.

Unlike the dry season, male representation varied significantly across the age groups, with the least of 22% in the 31-40 age groups highest of 50% in the 0-10 age group (Figure 4). RDT positive prevalence also varied significantly by age group, from the least risk of 13% in the 40 years plus age group to a highest of 38% in the 11-20 year group. Malaria parasite prevalence also followed the same trend. The 0-10 year group had the highest reported ITN use the night before survey (about 26%) (Table 4.6).

4.1.3 Risk Ratios

Adjusted (for confounding) risk ratios were estimated by zone and age groups, and are presented in Table 4.6.

Risk ratios for both zones and age groups varied by season. Whiles Osudoku significantly had half the risk of malaria infection as that of Dodowa in the wet season, the risk of infection almost equalled out in the in the dry season. Prampram zone showed no seasonality in risk of infection. For all zones, the adolescent group bore the highest risk for malaria infection for both seasons, which was marked in the dry season.

Table 4.7. Adjusted Risk ratios by season, zone and age group with p-values.

Category	Wet Season		Dry Season	
	Adjusted Risk ratio, (95%CI)	*p*-value	Adjusted Risk ratio, (95%CI)	*p*value
Malaria Risk Ratios by Zone				
Dodowa	1		1	
Osudoku, Dodowa	0.46 (0.24-0.9)	0.023	0.78 (0.52-1.17)	0.223
Prampram, Dodowa	1.1 (0.71-1.74)	0.63	1.08 (0.73-1.60)	0.69
Malaria Risk Ratios by Age group				
0-10 years	1		1	
11-20 years	1.3 (0.44-0.7)	0.44	1.49 (1.0 -2.21)	0.05
21-30 years	0.35 (0.12-1)	0.05	1.01 (0.54-1.92)	0.96
31-40 years	0.37 (0.14-0.99)	0.05	0.96 (0.51-1.84)	0.91
40-97 years	0.35 (0.22-0.56)	<.0.0001	1.07 (0.61-1.86)	0.86

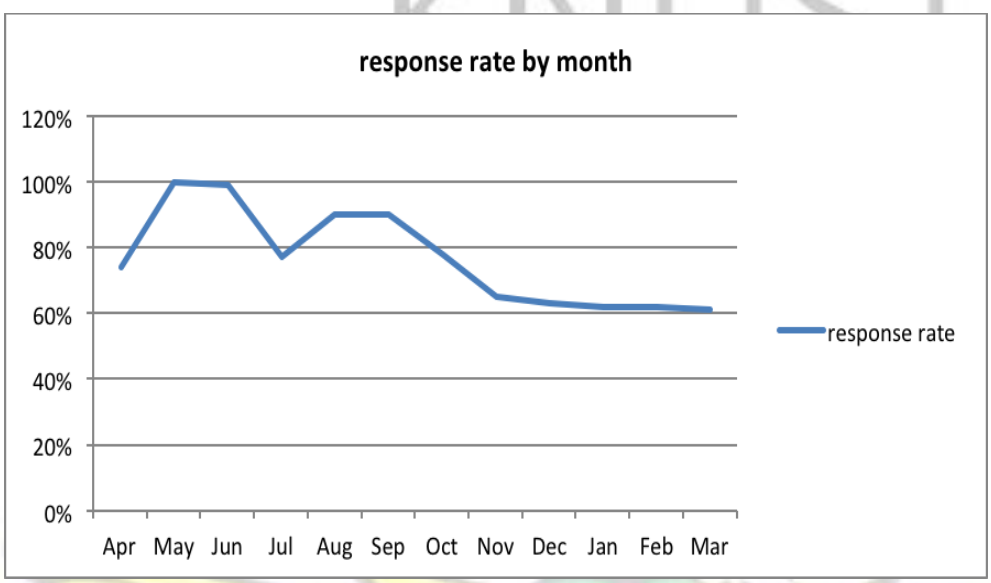
Risk ratios were using logistic regression modelling and adjusted for the confounding effects of area on age group measures and age group on area measures. For zonal risk ratios Dodowa was used as the Baseline whiles the 0-9 year group was used as the baseline for the age group risk ratios. Ratios are presented with 95% confidence limits with coresponding p-values. Osudoku had the least malaria risk ratio for both seasons more marked in the wet season. **4.2 Malaria Incidence (Cohort Study)**

4.2.1 General Study Description

The overall response rate was 79% (completed anticipated visits). Starting with 1950 participants, the response rate peaked in the second month with just over 3000 participants. This included all newborn babies who were not sampled but were rolled in to the cohort in the communities at identification of participants. It then dipped in

the rainy season (June and July). This dropped gradually, stabilizing at about 60% towards the dry season (November to March) (Figure 4.1).

Figure 4.1: Response by participants by month over the study period N=36941 (total planned visits)



Proportions of successfully completed visits to planned visits by month are presented. Total completed visits =28543(79% N). The study initiation month of April recorded the least number of completed visits mainly due to difficulties in tracing participants to their current locations, and inexperience of field workers in participant tracing. As the months progressed, completed visits improved but dropped down to about 60% in the dry season (from November) largely due to migration out of the district for farming and fishing activities.

The study population was similar in all three zones; the proportion of males was about 49.5% through all the zones (49.4% for Forest, 49.2% for Lakeside and 49.9% for Coastal zones). The study covered all age groups. The youngest was a day old and the oldest 97 years old. Participants aged 0-19 years constituted almost 50%. We completed almost 79% of all scheduled follow up visits. The overall history of fever in the last two weeks prevalence was 7%, out of which 30% tested positive for *Plasmodium falciparum* by RDT (Table 4.8). August recorded the highest history of fever in the last

two weeks prevalence of 14% with February and March having the least prevalence of 1% each.

The study covered all age groups to provide a complete characterized malaria epidemiology of the district and serve as baseline for further tracking of progress as malaria control efforts improve, and scale up through elimination to eradication. The contribution of each age group in person years was also similar, 0 to 9 years contributed 336.1 person years, 10 to 19 years 385.4, 20 to 29 years 329, 30 to 39 years 340.5 and above 40 years 414.7 person years.

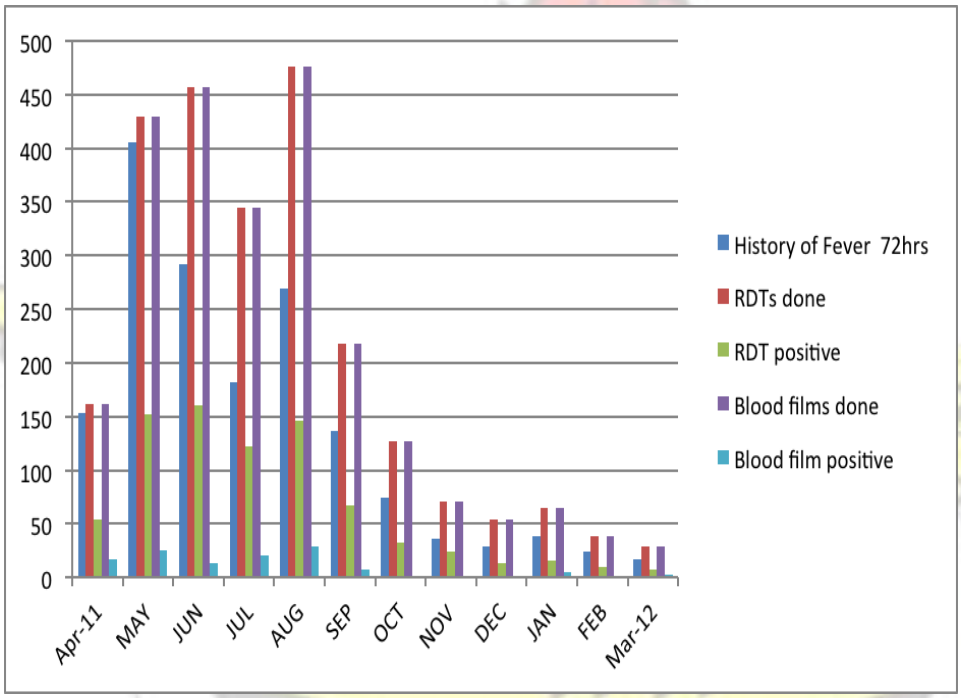
Table 4.8: General Description of study and Participant characteristics N=36164.

Month	Apr-11	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar12	
Round	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**Total**
# sampled	1950 (502ex)	3181	3181	3181	3181	3181	3181	3181	3181	3181	3181	3181	36164
# participants (N)	1448 (100%)	3268 (100%)	3156 (99%)	2457 (77%)	2871 (90%)	2871 (90%)	2489 (78%)	2075 (65%)	2005 (63%)	1985 (62%)	1977 (62%)	1941 (61%)	28543 (79%)
History of Fever 2/52	161 (11%)	400 (12%)	372 (12%)	279 (11%)	391 (14%)	182 (6%)	91 (4%)	52 (3%)	38 (2%)	53 (3%)	23 (1%)	22 (1%)	2064 (7%)
History of Fever 72hrs	153 (11%)	405 (12%)	292 (9%)	182 (7%)	269 (9%)	136 (5%)	74 (3%)	36 (2%)	29 (1%)	39 (2%)	24 (1%)	17 (1%)	1656 (6%)
RDTs	162	430	457	344	476	218	127	71	54	65	38	29	2471
#RDTs Indeterminate	1	4	0	5	4	4	12	1	3	1	2	2	39
RDT positive	54 (34%)	152 36%	160 35%	122 36%	146 31%	67 31%	33 29%	24 34%	13 25%	16 25%	10 28%	8 30%	805 33%
Blood films (BF)	162	430	457	344	476	218	127	71	54	65	38	29	2471 (100%)
BF positive	17 (10%)	25 (6%)	13 (3%)	21 (6%)	29 (6%)	7 (3%)	1 (1%)	2 (3%)	2 (4%)	5 (8%)	0	3 (10%)	125 (5%)

Analysis was done on only completed visits, n=28543(79%N) and are presented in frequencies and proportions. Prevalence of history of fever in the past two weeks peaked in August at 14% with the lowest prevalence occurring in the February and March (1%) with an average annual prevalence of 7%. 33% of all those who reported fever within the last two weeks tested positive for RDTs and 5% for malaria parasite by microscopy.

RDT and blood film tests were performed for those who responded positively to having fever now (that is on the day of visits), fever in the last 72 hours or fever in the last 2 weeks. The variation in numbers who responded to having fever in the past 72 hours and their corresponding RDT and Blood smear results by month are represented in the figure 4.2.

Figure 4.2: History of Fever in the past 72 hours with corresponding RDT and blood smear results for all zones by month



Frequencies are presented by month. RDTs and Blood smears were done for both history of fever in the past 72 hours and fever in the past 2 weeks. Prevalence of reported fever peaked in May.

The wet season, from April to September recorded the highest numbers for perceived fever and RDT positivity (increased likelihood for perceived fever to be malaria) than in the dry season.

The level of verified ITN use the night before home visits was very low generally, worse in Dodowa (3.3%), compared to the national and regional averages of 28.6% and 10.9% respectively, which were not verified. The Indoor Residual Spraying (IRS) coverage was very low for the district (1.4%), but compares favorably with the regional average of 1.5% (Ghana Statistical Service 2012), Dodowa had the highest IRS coverage of 5.6%, which is over four times the district average of 1.4%. Dodowa with the highest number of health facilities and pharmacy outlet had the highest (three fold) access to ACTs for perceived fever (6.1%) compared to Osudoku (1.9%) and Prampram (2%) (Table 4.9). Participants from Dodowa travelled out of their communities in the two weeks before visits (13%) and had more farms located within 30meters radius of their houses (35%) than those from Prampram (1%, 6%)and Osudoku (7%, 12%).

Table 4.9: General characteristics of participants (weighted) by zone n=28543 visits

Zone	Dodowa (Forest)	Osudoku (Lakeside)	Prampram (Coastal)	Total	*p*-values
Total person years of follow up	565.3	610.2	603.3	1778.7	
% Male	49.4	49.2	49.9	49.5	
% Fever Since Last 2 Week	8.76	6.80	8.0	8.02	0.3590
% Anti-malarial use for perceived fever	6.1	1.88	2.04	3.34	<0.0001
% Lab test use before anti-malarial use	1.52	0.57	0.62	0.81	0.0022
% Presence of ceiling in the room	24.43	9.47	4.9	10.97	0.0002
%Open Water body within 30m radius of house	12,4	24.5	46.4	30.8	0.0200
%Animal Enclosure within 30m of house	53.7	57.1	39.2	48.9	0.3400
%Presence of Farm within 30m radius of house	34.8	11.6	6.3	17.2	<0.0001
%ITN use last night	3.3	8.7	4.1	5.6	0.002
%IRS use	5.6	0.3	0.0006	1.4	0.0001
%Travel outside the community for more than a day since last 2weeks	12.7	7.4	0.8	5.9	0.0001

Analysis was done by Cox regression. Cohort was ordered by follow up time, proportions are presented with p-values. Dodowa had the highest access to testing and antimalarial drugs with Osudoku having about a third of the Dodowa levels.

4.2.2 Incidence Rates

The rate analysis was done two ways, fever now or within the last two weeks plus RDT positive and fever in the last two weeks plus blood smear by microscopy positive.

Table 4.10: Variation of Malaria incidence rates (weighted crude rates) by month, RDT malaria events for all zones

Month	Person years	#RDT malaria events	Incidence Rate/1000 person years
April	1.1	54	49090
May	123	141	1146

June	155.5	147	945
July	144.6	107	740
August	160.4	137	854
September	190.9	62	325
October	162.9	30	184
November	162.3	17	105
December	134.3	8	60
January	213	13	61
February	151.8	6	40
March	178.9	7	39
Annual	1778.7	729	410

May recorded the highest crude incidence rate (about 1150 per 1000py) with March recording the least of 39per 1000py). April had the least person years of follow up due largely to ineffective participant tracing at the initiation of study.

The month of May had the highest incidence of malaria, decreasing to the least burden in March for all the zones (Table 4.10).

RDT overestimated microscopy-defined malaria events nine fold (729 to 80) for the overall total events and crude incidence rates (410 to 45). Whether by RDT or microscopy, Dodowa had the highest crude incidence rates, with about twice the microscopy defined incidence rate (85 per 1000 person years), then Prampram (41 per 1000 person years), with Osudoku having the least incidence rate (13 per 1000 person years). The differences were statistically significant (Table 4.11)

Table 4.11: Crude Rates with Mantel-Haenszel rate ratios, by zone

Zone	Person Years	#BF Malaria events	BF Rate/ 1000py (95%CI)	BF Rate Ratios (MH) (95%CI)	*p*-value	#RDT + fever 2/52 events	RDT rate/ 1000py (95%CI)
Osudoku (Lakeside)	610.2	8	13.1 (7-26)	1		145	238 (202-280)
Prampram (Coastal)	603.3	25	41 (28-61)	3.16 (1.43-7.01)	0.003	275	456 (405-513)

Dodowa (Forest)	565.3	47	85 (63-111)	6.63 (3.0-13.42)	<0.001	309	547 (489-611)
Total	1778.7	80	45 (36-56)			729	410 (381-441)

Rates and Rate Ratios are presented with 95% confidence limits with corresponding p-values. Osudoku rates were used as baseline for the Mantel-Haenszel rate ratios. Both Microscopy and RDT defined rates are presented. Osudoku had the least malaria parasite incidence rate ratio, Prampram had a threefold rate ratio as Osudoku, and Dodowa had a sevenfold rate ratio as Osudoku.

Analysis by both Blood smear positive with history of fever in the last two weeks and RDT Positive Malaria, (fever in the last 2 weeks +Positive blood film or RDT) are presented in Table 4.11. RDT estimates overestimate malaria in Dodowa six (6) fold and an excess risk of 462/ 1000py, eighteen (18) fold in Osudoku with an excess risk of 225/100py and ten (10) in Prampram with a malaria excess risk of 365/100/py. This is so because RDT positivity reflects the prevalence of malaria infection in the preceding 2-4 week (effectively a period prevalence), whereas microscopy provides an instantaneous prevalence of malaria parasite infection.

4.2.3. Relative Risks

Weighted and adjusted relative malaria risks with Osudoku as baseline are shown in Table 4.12.

Table 4.12: Malaria Incidence Rate ratios by Zone by RDT defined rates, with 95 percent confidence limits and corresponding *p*-values

	Rate Ratio (95%CI)	***p*-values**
Total District, Osudoku	**Dodowa, Osudoku**	
	0.902 (0.824-0.968)	0.235
	-2.802)	<0.0001
2.3 (1.889		
Prampram, Osudoku	1.918 (1.569-2.346)	<0.0001
Total District, adjusted for travel outside 2/52	0.936 (0.853-1.027)	0.1648
Total District, adjusted for presence of ceiling	0.853 (0.777-0.936)	0.0008

Analysis was done by Cox regression modeling and Osudoku rate used as baseline. Rate ratios by RDT incidence still had Prampram significantly having double the rate of Osudoku with Dodowa having 2.3 fold the rate of Osudoku.

Dodowa and Prampram had twice the risk of Osudoku, though Osudoku has vast irrigated fields. Travelling outside the district within two weeks of visit did not affect the risk of infection significantly, though the presence of a ceiling in the room where participant slept reduced the risk significantly.

Though the 5 to 9 year group had the highest rates for blood film positive events, the 0 to 4 year age group had the highest rates for RDT positive events.

Table 4.13: Crude Malaria Incidence Rates by age group

Age category, years	Person Years	# Events blood film positive	Rate/1000py blood film positive (95% CI)	# Events RDT positive	Rate/1000py RDT (95% CI)
0-4	167.6	20	119 (77-185)	138	823 (697-973)
5-9	168.5	23	136 (91-205)	136	807 (682-955)
10-19	385.4	18	50 (32-79)	164	458 (393-533)
20-29	329	3	9 (3-28)	81	246 (198-306)
30-39	340.5	6	18 (8-39)	96	282 (231-344)
40-97	414.7	10	24 (13-45)	114	275 (229-330)

Crude but weighted incidence rates are presented with 95% confidence limits with corresponding p-values. Both Microscopy and RDT defined rates are presented. For both RDT and Malaria parasite positive incidence rates, the 0-9 year group had the highest rates.

Expectedly, the 0-4 year had the highest crude incidence, but 5-9 year olds had almost the same rate as the 0-4 year olds (Table 4.13).

Table 4.14: Crude RDT and Malaria Parasite Incidence Rates by age

Age category, years	Rate/1000py Microscopy positive	Rate/1000py RDT Positive	Rate Ratio, RDT/Microscopy	Excess Risk, RDT-Microscopy/1000py
0-4	119	823	6.9	704
5-9	136	807	5.9	671
10-19	50	458	9.2	408

20-29	9	246	27.3	237
30-39	18	282	15.7	264
40-97	24	275	11.5	251
Total	45	410	9.1	365

For both RDT and Malaria Parasite rates, the 0-9 year had the highest crude incidence, with the 20-29 year old group having the least

Across all age groups, RDT defined incidence rates, which reflects the period prevalence expectedly was higher than the malaria parasite incidence, the least being in the 5-9 year olds and more marked in the 20-29 year group (Table 4.14). Expectedly, the 0-4 year had the highest crude incidence, but 5-9 year olds had almost the same rate as the 0-4 year olds.

When the crude weighted rates ratios were adjusted by hazards assumption in Cox regression, the differences observed in the crude rates persisted and were statistically significant as shown in the Table 4.15.

Table 4.15: Incidence hazard ratios (RDT defined Malaria), by zone and age group.

Category	Hazard ratios, (95%CI)	*p*-value
Dodowa	1	
Osudoku, Dodowa	0.46 (0.38-0.56)	<.0.0001
Prampram, Dodowa	0.89 (0.75-1)	0.144
0-4 years	1	
5-9 years	0.97 (0.77-1.2)	0.81
10-19 years	0.55 (0.44-0.7)	<.0.0001
20-29 years	0.31 (0.23-0.4)	<.0.0001
30-39 years	0.35 (0.27-0.45)	<.0.0001
40-97 years	0.34 (0.26-0.43)	<.0.0001

Dodowa and the 0-4 year old group served as baselines. Analysis was by Cox regression modeling (Hazards assumption) and ratios are presented with 95%

confidence limits and corresponding p-values. The rate ratios were the same as estimated by the Mantel-Haenszel method.

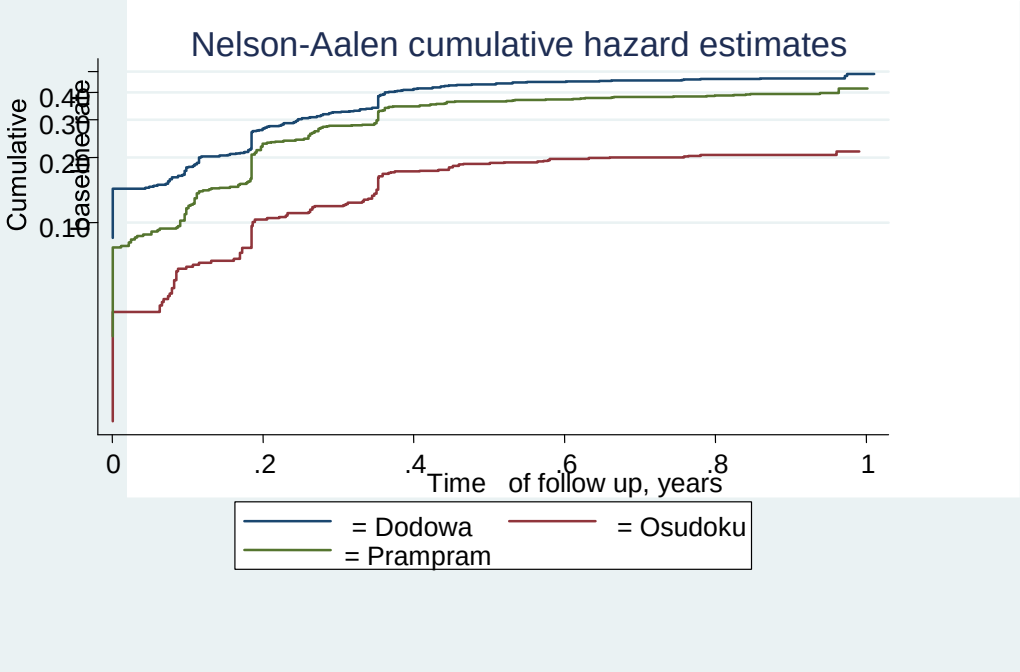
Hazard ratios, by RDT defined malaria followed the same pattern. Prampram zone had the same ratio as Dodowa, but Osudoku zone had half the risk of Dodowa.

4.2.4 Cumulative Incidence by Zone and Age

The Figure 4.3 shows the cumulative incidence rates over the time of follow up, showing changes in hazard ratios over very small changes in time for the three zones. It shows the estimated number of malaria cases per unit time at risk. Dodowa (Forest) and Prampram (Coastal) had similar rates throughout the year with Osudoku (Lakeside) having a significantly lower risk.

Adjusted Cumulative malaria incidence showed proportionality and closeness of the Dodowa and Prampram plots which do not separate over time of follow up, suggesting no increasing effect over time of follow up between the two zones and therefore no significant difference in cumulative incidence, where as that of Osudoku stayed separate from Dodowa and Prampram

Figure 4.3: Cumulative incidence rates by zone over time of follow uP.



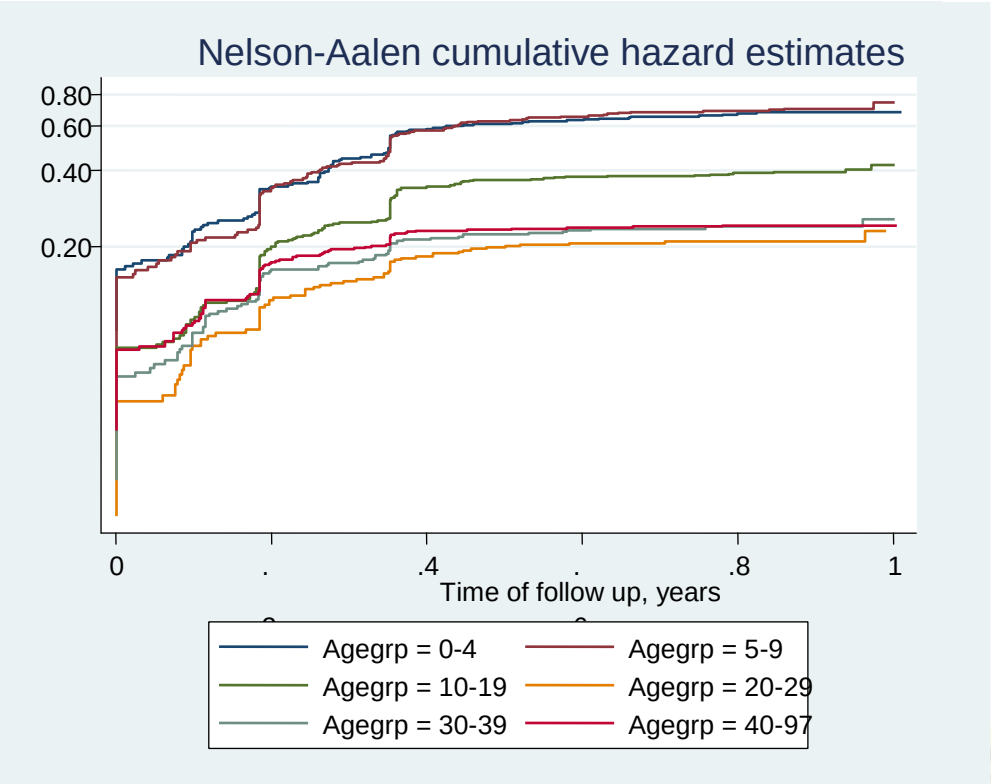
Analysis was done using cox regression (hazard assumption). Dodowa and Prampram had almost the same distribution curve with Osudoku having a lower area under the cumulative incidence curve throughout the time of follow up.

Similarly, the cumulative incidence rates were almost the same for Dodowa and

Prampram over the time of follow up, but both were significantly higher than

Osudoku rates throughout the period of follow up.

Figure 4.4: RDT malaria cumulative incidence rates by age groups over time of follow up



The 0 -4 year age group had almost the same distribution curve with the5-9 year old group, 10-19 year age group having a lower and 20 years and above having the least area under the cumulative incidence distribution curve throughout the time of follow up.

Cumulative incidence by age group showed the 0-4 and 5-9 year age groups having the highest incidence rates consistently throughout the year. The Hazard ratio showed no difference between the 0-4 and 5-9 age groups over the period of follow uP. The 10-19 age group had almost the same cumulative incidence rates as the 20-29, 30-39 and 40-97 year groups at beginning of follow up, but increased significantly after three months (after June) of follow up (Figure 4.4).

4.3: Entomology

4.3.1: Climatic Data

Rainfall and temperature results from a Ghana Meteorological substation in each of the three zones are presented. The Forest zone was collected from Afienya, Lakeside from Asutuare and Ada, was used as proxy for the Coastal zone. The results are summarized below.

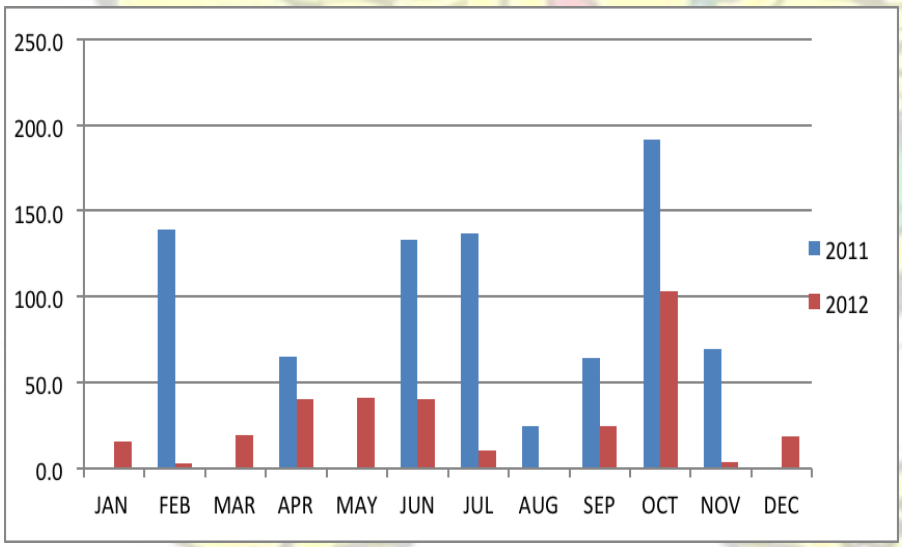
4.3.1.1 Rainfall

Rainfall in all three ecological zones followed a bimodal distribution with peaks in June, July and October.

4.3.1.1.1 Monthly Rainfall in the Forest Zone

Monthly rainfall for the Forest zone for both 2011 and 2012 followed bimodal distribution.

Figure 4.5: Forest Monthly Rainfall (mm) for 2011 and 2012



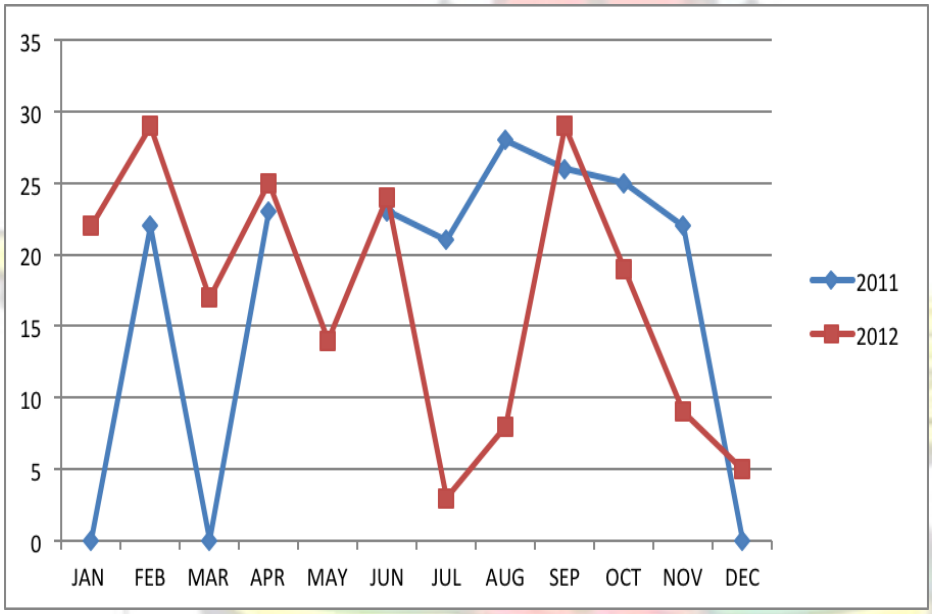
Data by courtesy of Ghana Meteorological Agency 2013

The maximum (peak) monthly rainfall was experienced in October for both years under observation (2011 (180.0 mm) and 2012 (100.0 mm) respectively). In contrast, the months of January, March, May, August and December in year 2011 recorded the lowest rainfall,

below 50.0 mm. Overall, 2011 recorded higher mean monthly rainfall than in 2012 (Figure 4.5).

Despite recording the lowest monthly rainfall, year 2012, in terms of the number of rainy days per month recorded the highest as indicated (in Figure 4.6) below; in February and September respectively. The maximum rainy days was observed in the month of September for the years 2011 and 2012 respectively (Figure 4.6).

Figure 4.6: Number of rainy days by month, Forest Zone for 2011 and 2012



Data by courtesy of Ghana Meteorological Agency 2013

4.3.1.1.2 Monthly Rainfall in the Lakeside Zone

The highest monthly pattern of rainfall for the Lakeside zone occurred in June (320.0 mm) and October (180.0 mm), for years 2011 and 2012 respectively. There was zero (0.0 mm) rainfall in the months of January, February, March and December in year 2011. In the year 2012, the months of January, August and September did not experience any rainfall as depicted in the **figure 4.7**.

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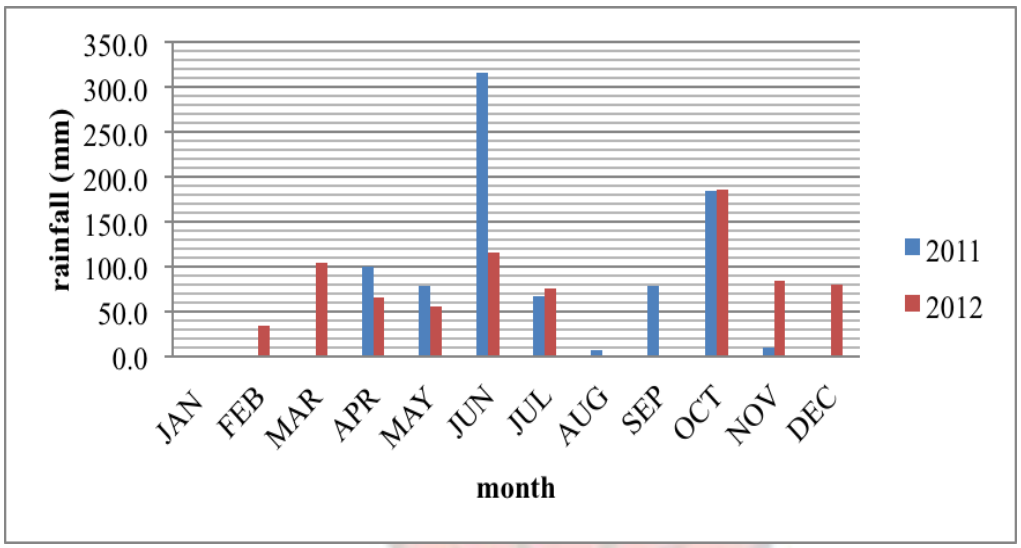
Figure 4.7 Lakeside Monthly Rainfall (Mm) for 2011 and 2012



Data by courtesy of Ghana Meteorological Agency 2013

Regarding the number of rainy days in the Lakeside zone, year 2012 recorded the highest number of days. It recorded 16 days of rain in the month of October, as opposed to 15 days of rain in year 2011 as shown in the **figure 4.8**.

The number of rainy days followed the same pattern for both 2011and 2012, with a bimodal distribution, with peaks at May/June for the main rainy season and October, the so-called minor rainy season. For 2012, October had more rainy days than May and June.

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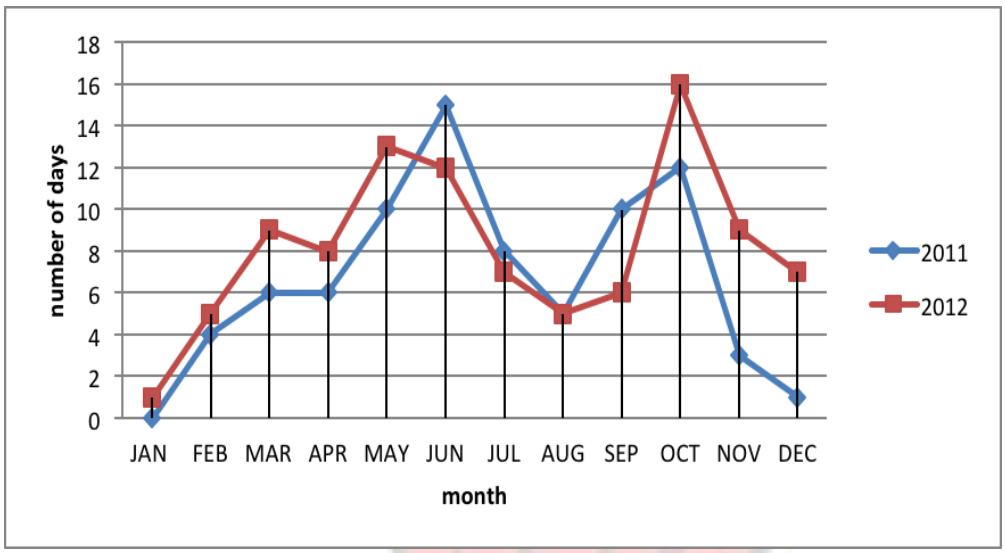
Figure 4.8 Lakeside number of rainy days per month for 2011 and 2012



Data by courtesy of Ghana Meteorological Agency 2013

4.3.1.3 Monthly Rainfall in the Coastal Zone

Like the pattern of monthly rainfall in the Lakeside zone in the year 2011, the coastal zone also recorded its monthly peak of rainfall in June (290.0 mm). The highest monthly rainfall pattern in year 2012 occurred in May, recording 150.0 mm of rainfall. Whereas in every month there was a least a millimeter of rainfall in year 2012, the month of January in year 2011 did not record any rainfall (zero (0.0 mm) rainfall) as displayed in **figure 4.9**

Figure 4.9 Coastal Mean Monthly Rainfall (mm) for 2011 and 2012.

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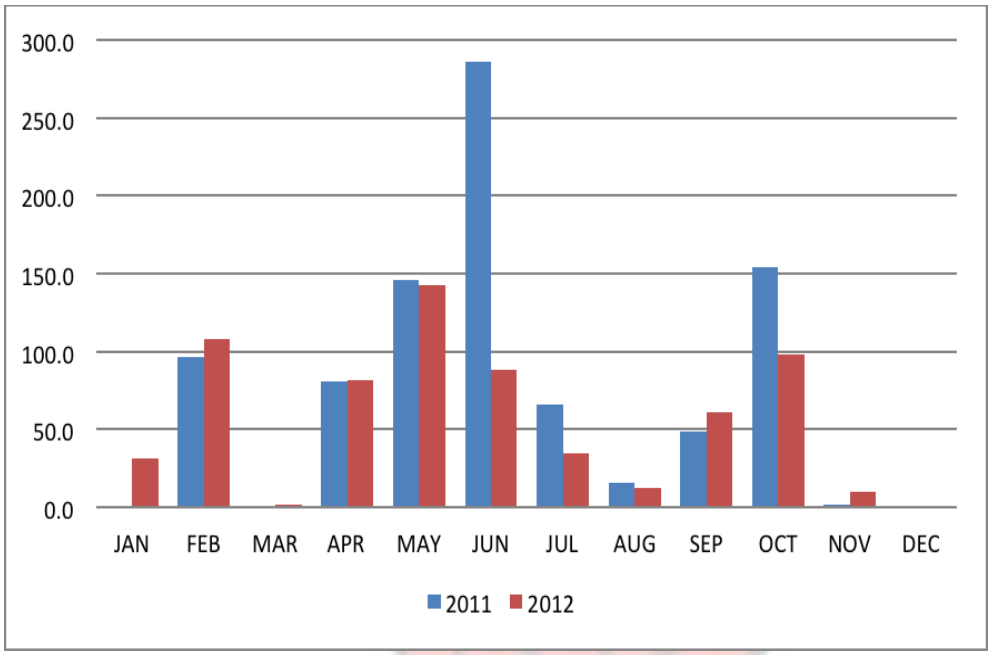
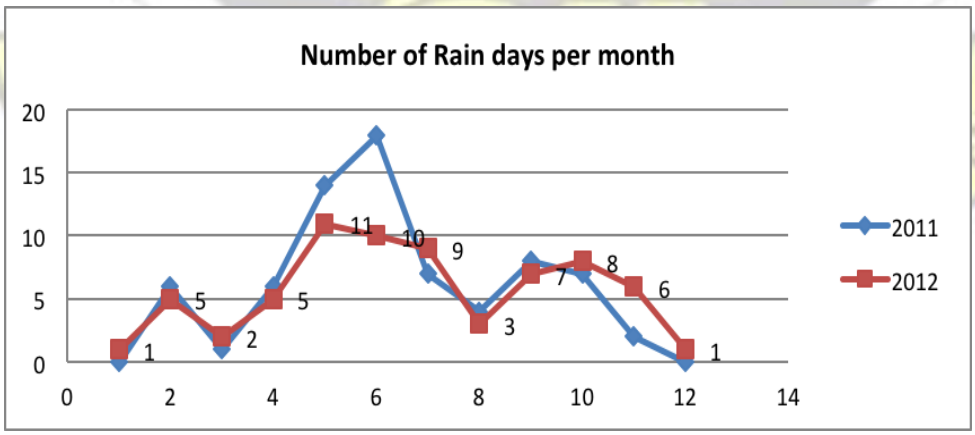


Data by courtesy of Ghana Meteorological Agency 2013

Contrary to the Lakeside zone experiencing a higher number of days of rain in year 2012, the Coastal zone experienced the higher number of rainfall days in year 2011, recording rains in about 18 days, compared to only 10 days in 2012 as shown in **figure 4.10**.

Figure 4.10: Coastal Number of rainy days per month for 2011 and 2012



Data by courtesy of Ghana Meteorological Agency 2013

The distribution of the number of rainy days for the Coastal zone also follows a bimodal distribution, the higher in June and the lesser mode in late September for both years under observation.

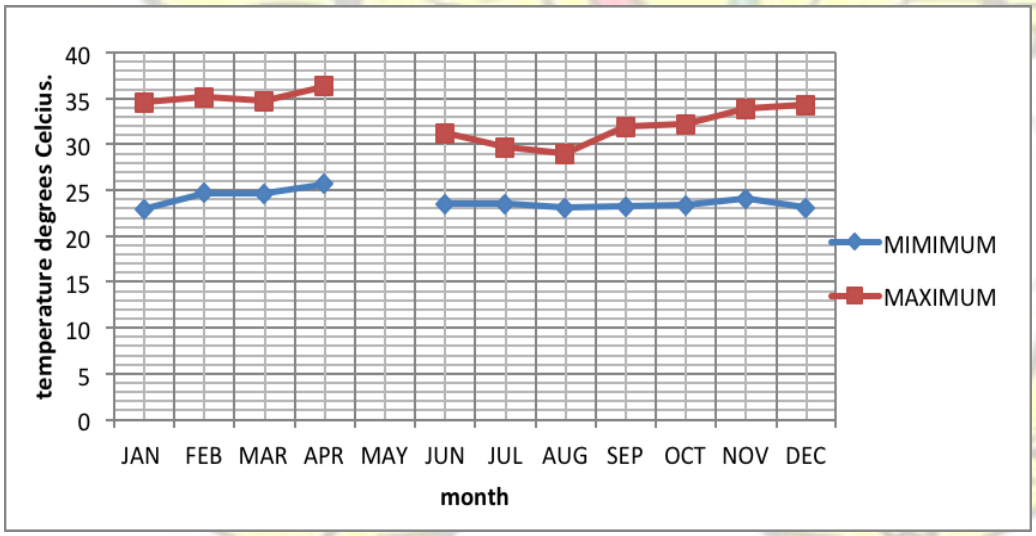
4.3.2 Temperature

The maximum and minimum temperature distribution for all the zones was similar for the period of observation.

4.3.2.1 Forest Zone Temperatures

No readings were obtained during the last week of April, May and first week of June 2011 due to the breakdown of equipment, which was replaced in the second week of June. These months corresponds to the main rainy season days (Figure 4.11).

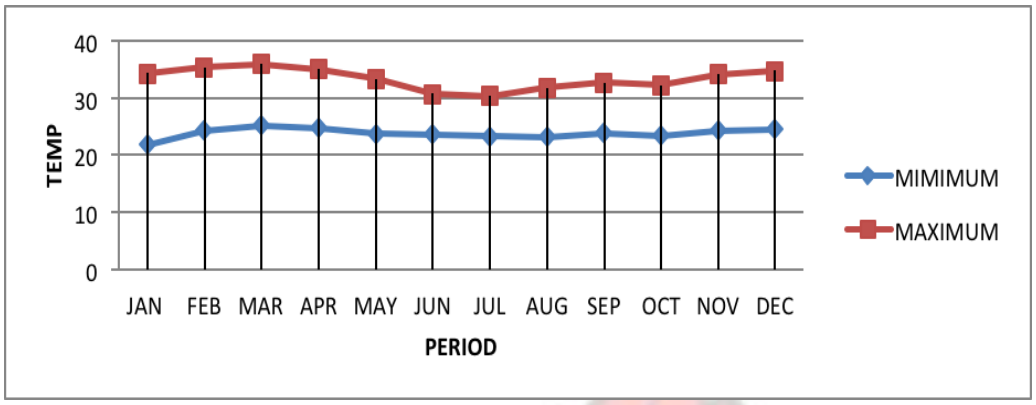
Figure 4.11: Forest Zone Maximum and Minimum Temperature (°C) by month for 2011



Data by courtesy of Ghana Meteorological Agency 2013

The 2012 data followed the same trend as the 2011 and is depicted in the figure 4.12.

Figure 4.12: Forest Zone Maximum and Minimum Temperature (°C) by month for 2012

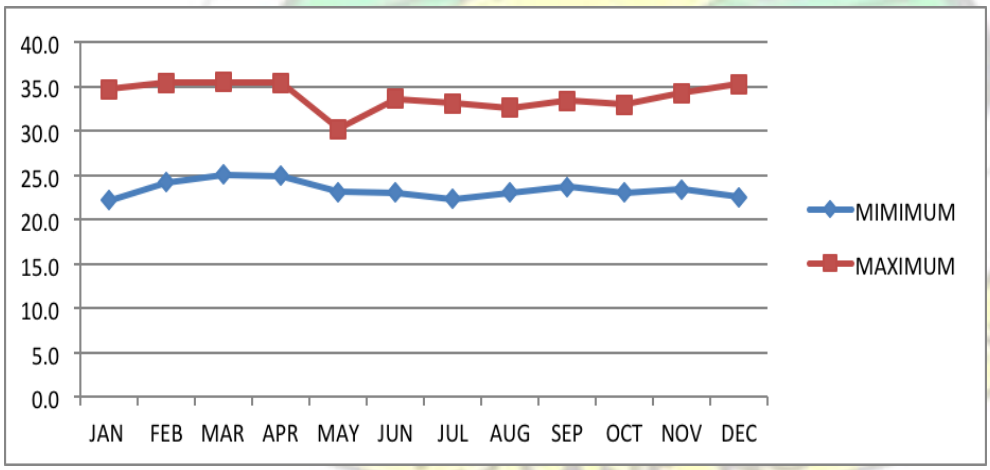


Data is by courtesy of Ghana Meteorological Agency 2013

4.3.2.2 Lakeside Zone Temperatures

Generally, the lakeside zone experienced the lowest maximum and minimum temperatures in May and peaks in December. The 2011 readings characteristically followed this trend (Figure 4.13).

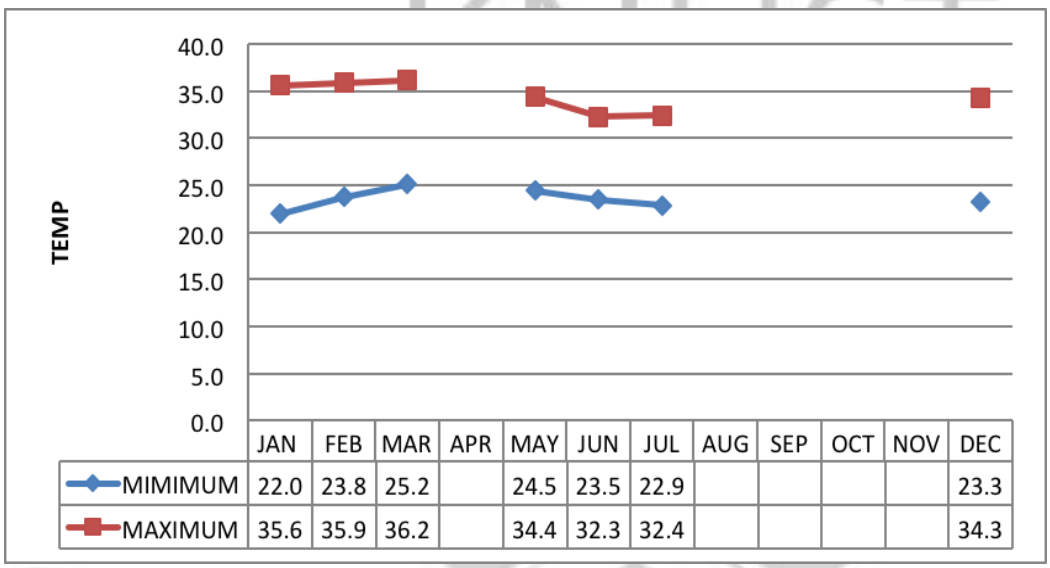
Figure 4.13: Lakeside Temperature, maximum and minimum (°C) by month for 2011



Frequencies are presented. Data by courtesy of Ghana Meteorological Agency The hot period corresponds to the dry season with both minimum and maximum temperatures increasing from November and peaking in March.

The 2012 minimum and maximum temperatures for the Lakeside zone followed the same trend as 2011, though missing some readings (Figure 4.14).

Figure 4.14: Lakeside Maximum and Minimum Temperature (°C) by month for 2012



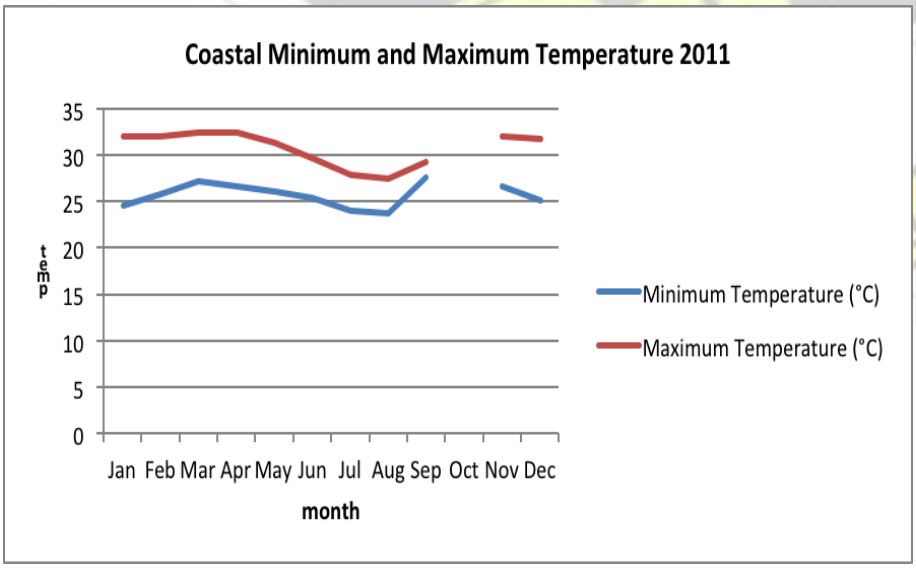
	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
MIMIMUM	22.0	23.8	25.2		24.5	23.5	22.9					23.3
MAXIMUM	35.6	35.9	36.2		34.4	32.3	32.4					34.3

Data is by courtesy of Ghana Meteorological Agency 2013

4.3.2.3 Coastal Zone Temperatures

The coastal zone had lowest maximum and minimum temperatures in July-August for both years under observation instead of May/June as observed in the Forest and Lakeside Zone (Figure 4.15)

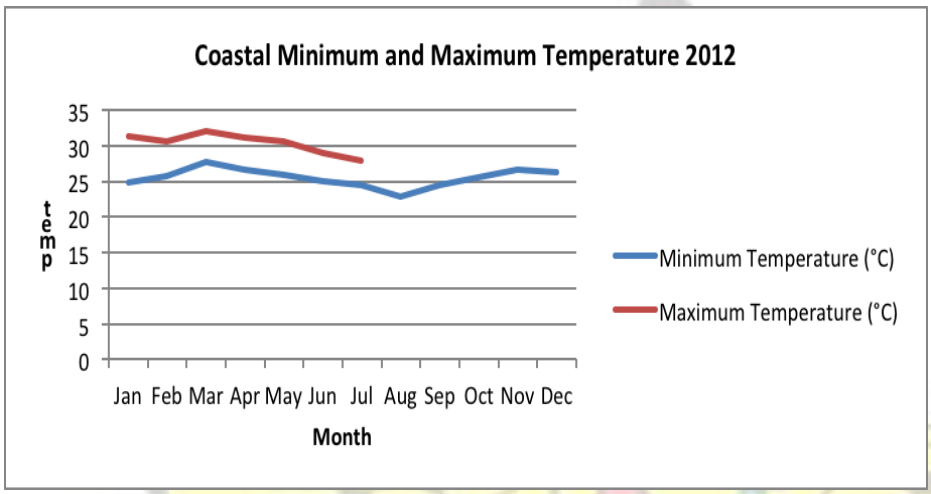
Figure 4.15: Coastal Maximum and Minimum Temperature (°C) by month for 2011



Data for October was unavailable. Data is by courtesy of Ghana Meteorological Agency 2013

In 2012, the coastal zone experienced the same trend for temperatures as in 2011 though some readings for maximum temperature are missing (Figure 4.16).

Figure 4.16: Coastal Maximum and Minimum Temperature (°C) by month for 2012



Maximum temperatures were unavailable for July to December. Data by courtesy of Ghana Meteorological Agency 2013

4.3.3. General Characteristics of Entomological catches

The total catches and Entomological Inoculation Rates (EIR) for the whole district is presented in Table 4.16. The highest occurred in April, which marked the beginning of the rainy season immediately following the end of intense dryness and heat of March, with an annual rate of 146 infective bites per person per year, though the most abundant catch of anopheles (276) occurred in May. Cullicine catch was highest in June with 609 and least in March with 11. The number of *An. gambiae* was about 19 times the catch of *An. funestus*. The proportion of catches positive for circumsporozoite protein (CSP) was highest (0.17) in January and lowest in October,

February and March. The man biting rate (MBR) per man night was lowest (0.0) in March and highest (10.6) in May. Out of the 616 tested, only 28 were positive. Two months (October and February) had catches that tested zero positives.

Table 4.16: General Characteristics, all zones, Data is presented by month

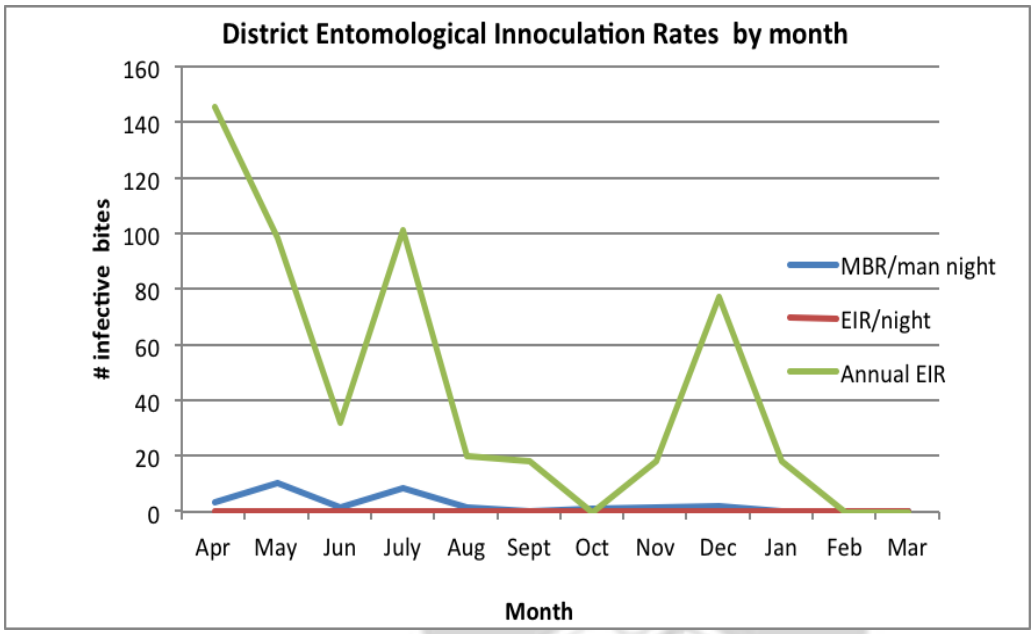
Month	*An. An.* total	#teste	# Proport	MBR/ EIR/	EIR/ cullici	*Gambi Funest*	Anoph d for	Posi ion man	Nigh
year	nes	*ae us*	eles	CSP	tive	positive	night	t for CSP	
Apr	51	14	65	65	8.	0.12	3.25	0.40	146.00	114
May	264	12	276	236	6	0.03	10.62	0.27	98.51	439
Jun	38	3	41	36	2	0.06	1.58	0.09	31.98	609
July	203	2	205	154	5	0.03	8.54	0.28	101.22	154
Aug	28	2	30	30	1	0.03	1.67	0.06	20.28	80
Sept	9	0	9	9	1	0.11	0.45	0.05	18.25	103
Oct	20	0	20	19	0	0.00	1.00	0.00	0.00	63
Nov	28	1	29	29	1	0.03	1.45	0.05	18.25	102
Dec	48	3	51	30	3	0.10	2.13	0.21	77.56	125
Jan	6	0	6	6	1	0.17	0.30	0.05	18.25	121
Feb	2	0	2	2	0	0.00	0.09	0.00	0.00	93
Mar	0	0	0	0	0	0.00	0.00	0.00	0.00	11
Total	**697**	**37**	**734**	**616**	**28**	**0.05**	**2.78**	**0.13**	**46.13**	**2014**

The Forest Zone followed the same trend, with maximum EIR occurring in April, and an average annual of 81 infective bites per year, twice the district average. The detailed monthly results follow in Table 19. The EIR per year was zero in six of the twelve months under study. These were August, September, October, January,

February and March. The EIR per night for the other 6 months was highest (0.8) in April. The highest total number of Anopheles catch (113) occurred in July. The proportion of catches positive for CSP was high (0.3) in April with zero proportion positive for CSP in above six months with zero EIR. The Man Biting Rate (MBR) per man night was lowest (0.0) in September and March and highest (14.1) in July. Out of the 331 tested, only 21 were positive. Four months (August, October, January and February) with catches and tested had zero positives.

The total District entomological rates for the period are represented in Figure 4.17.

Figure 4.17: District Entomological Inoculation rates by month April 2011 to March 2012

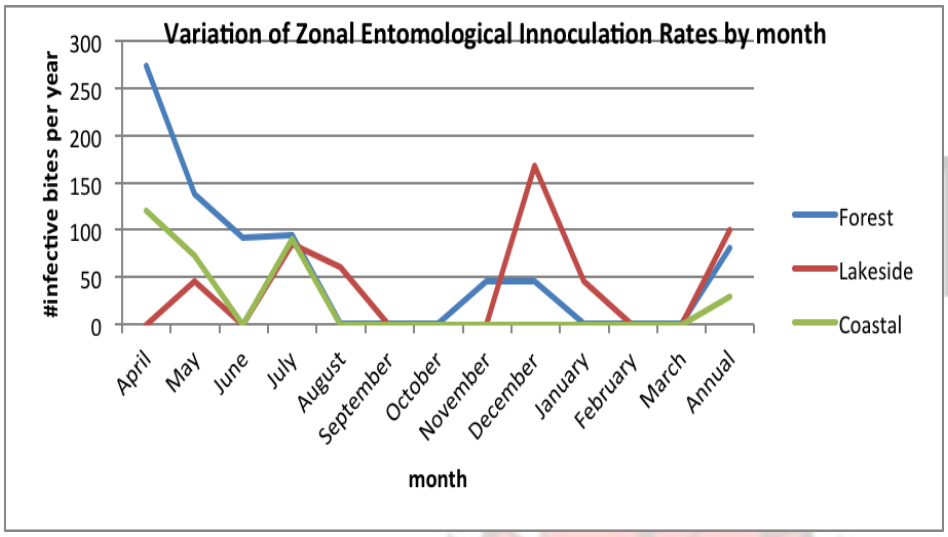


Frequencies are presented. The Man biting rate and EIR followed the same trend, and had three peaks in April, July and December.

4.3.3.1 Zonal Entomological Characteristics

The zonal entomological characteristics did follow the total district characteristics. Expectedly, the Osudoku, the Lakeside zone had the most abundance of mosquitoes, most of which were cullicines but with the highest percentage of mosquitoes positive for circumsporozoite protein and the least number of anopheles. The forest zone had the highest annual EIR, followed by the Lakeside with the Coastal zone having the least. For all the zones, the EIRs had double peaks in July and September (Figure 4.18).

Figure 4.18: Zonal Entomological Rates by Month from April 2011 to March 2012



Frequencies are presented. The zonal EIRs followed the same trend as the total district, with three peaks in April, July and December for all the zones.

4.3.3.1.1 Forest Zone Entomological Characteristics

The forest zone produced half the total anopheles catch for the whole district, 334/697(Tables 18, 19), with only 6% testing for circumsporozoite protein compared with 16% in the Lakeside zone and 3% in the coastal zone

Table 4.17: Entomological Rates per month, Forest Zone

Month	# Visits	Total Anopheles	# Tested	# Positive	Man Nights	Proportion positive	MBR per man night	EIR per night	EIR per month	EIR per year
Apr	4	24	24	6	8	0.25	3	0.75	22.5	273.7
May	4	79	79	3	8	0.04	9.88	0.38	11.4	136.8
Jun	4	23	23	2	8	0.09	2.88	0.25	7.5	91.25
Jul	4	113	110	2	8	0.02	14.13	0.26	7.8	93.74
Aug	3	12	12	0	6	0	2	0	0	0
Sep	4	0	0	0	8	0	0	0	0	0
Oct	3	19	19	0	6	0	3.17	0	0	0
Nov	4	28	28	1	8	0.04	3.5	0.13	3.9	45.63
Dec	4	8	8	1	8	0.13	1	0.13	3.9	45.63
Jan	4	3	3	0	8	0	0.38	0	0	0
Feb	2	1	1	0	4	0	0.25	0	0	0
Mar	4	0	0	0	8	0	0	0	0	0
Total	48	334	331	21	96	0.06	3.48	0.22	6.6	80.57

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8

Data is presented by month. Analysis was done by frequencies and proportions. April had the highest EIR per night of 0.75 with August, September, October, January, February and March recording zero infective bite per night.

4.3.3.1.2 Lakeside Zone Entomological Characteristics

The Lakeside zone entomological indices were as summed up in Table 20 below. Similar to the EIR occurring in the whole district and the forest zone, the lakeside zone also registered maximum EIR occurring in December, and an average annual of 100 infective bites per year, more than twice the district average, thrice that of the coastal zone and 20% more that of the forest zone. The EIR per year again, was zero in seven of the months under study similar to the forest zone. These were April, June, September, October, November, February and March. The EIR per night was highest

(0.5) was in December. The highest (55) total number of Anopheles catch occurred in May followed by July, with zero (0) catches in April, November and March. The proportion of catches positive for CSP was highest (0.3) in January. MBR per man night was lowest (0.0) in April, November and March, and highest (6.9) in May.

Table 4.18: Lakeside Zone entomological characteristics by month

Month	# Visits	Total Anopheles	# Tested	# Positive	Man Nights	Proportion positive	MBR/ man night	EIR/ night	EIR /month	EIR/ year
Apr	3	0	0	6	6	0	0	0	0	0
May	4	55	55	1	8	0.02	6.88	0.13	3.9	45.63
Jun	5	12	12	0	10	0	1.2	0	0	0
Jul	4	43	23	1	8	0.04	5.38	0.23	6.9	85.3
Aug	3	7	7	1	6	0.14	1.17	0.17	5.1	60.83
Sep	3	6	6	1	6	0	1	0	0	0
Oct	3	2	2	0	6	0	0.33	0	0	0
Nov	3	0	0	1	6	0	0	0	0	0
Dec	4	37	20	2	8	0.1	4.63	0.46	13.8	168.81
Jan	4	3	3	1	8	0.33	0.38	0.13	3.9	45.63
Feb	4	1	1	0	8	0	0.13	0	0	0
Mar	4	0	0	0	8	0	0	0	0	0
Total	**47**	**166**	**129**	**20**	**94**	**0.16**	**1.77**	**0.27**	8.1	**99.93**

Data is presented by month. Analysis was done by frequencies and proportions. December recorded the highest EIR of 0.46 infective bites per night, whilst April, Jun, September, October, November, February and March recorded zero infective bites per night.

4.3.3.1.3 Coastal Zone Entomological Characteristics

The results for the Coastal zone are as displayed in Table 4.19

Table 4.19: Entomological Rates, Coastal Zone

Month	# Visits	Total Anopheles	# Tested	# positive	Man Nights	Proportion positive	MBR/man night	EIR/ night	EIR/ month	EIR /year
Apr	3	41	41	2	6	0.05	6.83	0.33	9.9	121.67
May	5	102	102	2	10	0.02	10.2	0.2	6	73
Jun	4	10	10	0	8	0	1.25	0	0	0
Jul	4	21	21	2	8	0.1	2.63	0.25	7.5	91.25
Aug	3	11	11	0	6	0	1.83	0	0	0
Sep	4	3	3	0	8	0	0.38	0	0	0
Oct	3	0	0	0	6	0	0	0	0	0
Nov	4	1	1	0	8	0	0.13	0	0	0
Dec	4	3	3	0	8	0	0.38	0	0	0

Jan	3	0	0	0	6	0	0	0	0	0
Feb	4	1	1	0	8	0	0.13	0	0	0
Mar	4	0	0	0	8	0	0	0	0	0
Total	48	234	234	8	96	0.03	2.44	0.08	2.4	30.42

April recorded the highest EIR of 0.3 infective bites per night with the months August to March recording zero infective bites per night

The coastal zone recorded the lowest EIR in April, and an average annual of 30 infective bites per year; about one-third of the district's average as shown in Table 21 above. It recorded the lowest annual EIR compared to the forest and lakeside zones.

The annual EIR for the coastal zone occurred in three of the months under study. These were April, May, and July. The highest (102) total number of Anopheles catch occurred in May followed by April, with zero (0) catches in October, January and March during the study. The proportion of catches positive for CSP was highest (0.2) in July. MBR per man night was highest (10.2) in May. Six of the months had catches that tested zero positives for circumsporozoite protein.

4.3.4. Entomological Inoculation Rates

Anopheles were most abundant in May following the early rains in April, and decreased to almost zero levels in March, reflecting the rainfall patterns.

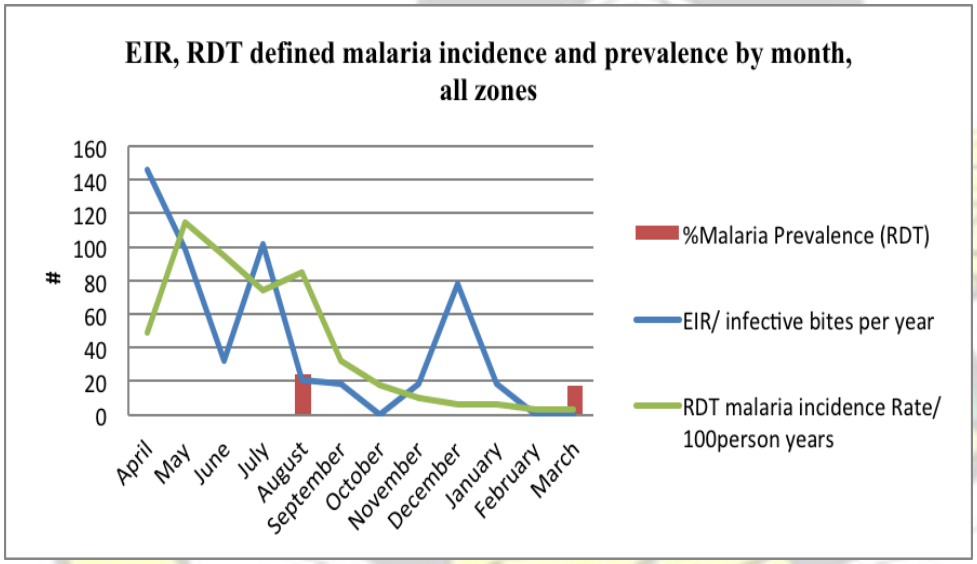
The Lakeside zone recorded the most abundant of Anopheles and the highest EIR of all with the Coastal zone having the least (a third of the Lakeside. The annual EIR for the district followed three peaks, the highest in May, followed by July and December Entomological inoculation rates and malaria incidence rates variation by month through the period of study for the district did not follow the expected trends. Peaks in incidence rates lagged behind Entomological inoculation rates by about a month. Also, whiles the entomological inoculation rates showed three peaks, the incidence rate followed only two peaks, which occurred in May and August. The malaria prevalence also did not

vary seasonally, showing no significant difference between the end of wet and dry season prevalence. It also did not vary with the Entomological inoculation rates.

4.4 Entomological Inoculation Rates, Malaria Prevalence and Incidence Rates

Overall district seasonal variation of EIR, Malaria prevalence and incidence rates are presented. Generally the largest seasonal fluctuations were observed in the entomological inoculation rates, followed by incidence rates whilst malaria prevalence remained stable by season (Figure 4.19).

Figure 4.19: Entomological Inoculation rates (EIR), RDT defined malaria incidence and malaria prevalence by month, all Zones



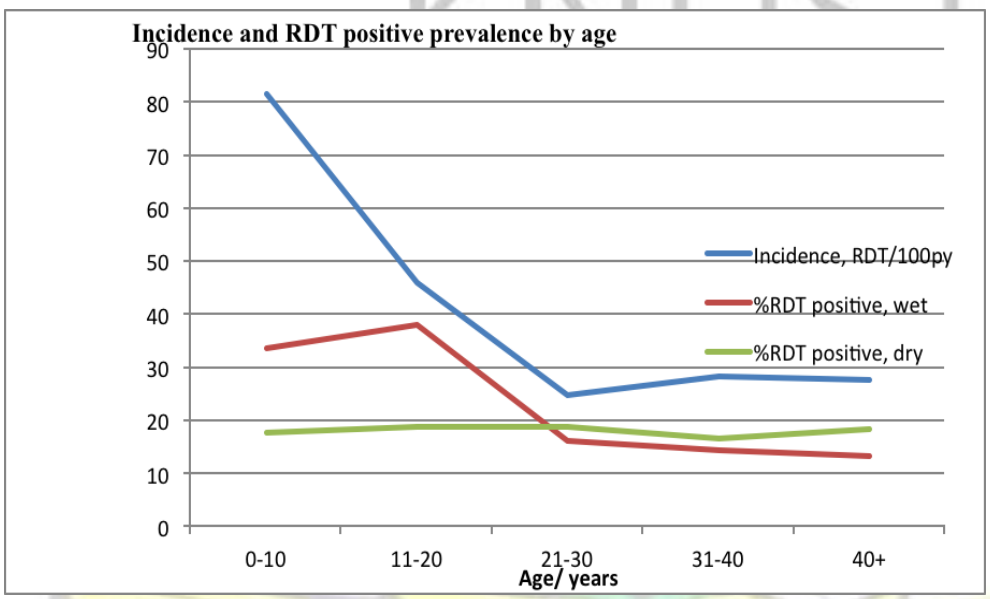
EIR and Incidence rates followed the same trend, a bimodal distribution with peaks in May and August for incidence rates, and July and December for EIR. Prevalence rates showed loss of seasonality

4.4.1 Malaria risk by age, overall district risk analysis.

Malaria risk and incidence rates are highest at the initial stages of life, the burden decreasing and stabilizing by age twenty years and staying at that level. Whiles the risk of malaria was almost even between 0-20 years and >20 years age groups in the dry

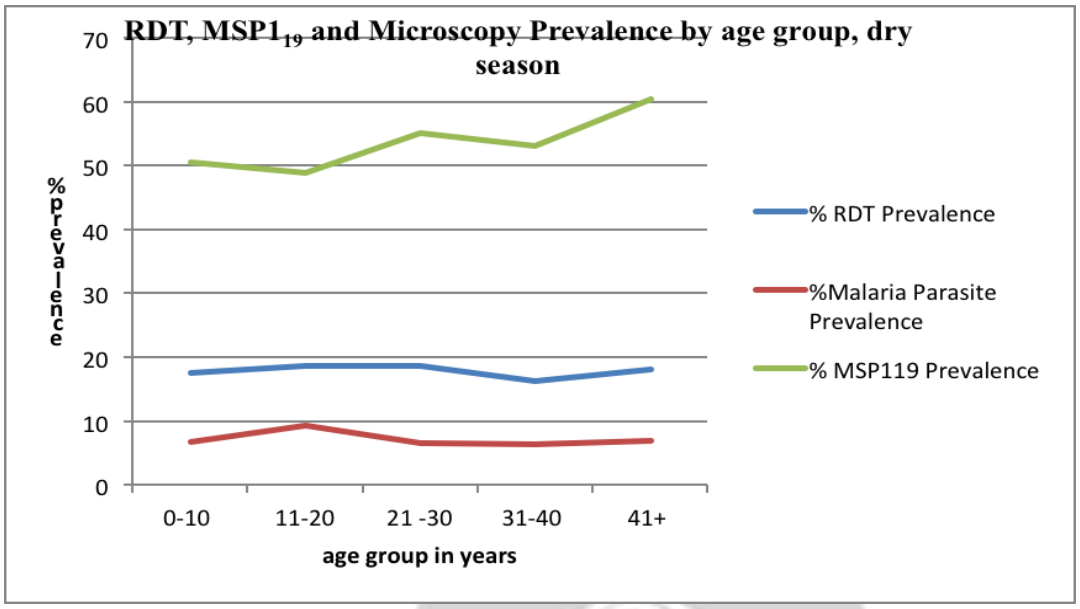
season, wet season difference was marked with the 0-20 year group bearing a higher risk (Figure 4.20).

Figure 4.20: Malaria (RDT defined) incidence rates and prevalence by age, all zones



Both incidence rates and prevalence decreased from the initial rates in infancy and plateaued at age 20, maintaining low levels from then on (Figure 4.20).

Figure 4.21: RDT positive, MSP-1_{19} positive and Malaria Parasite Prevalence by age, for the Dry Season, All zones

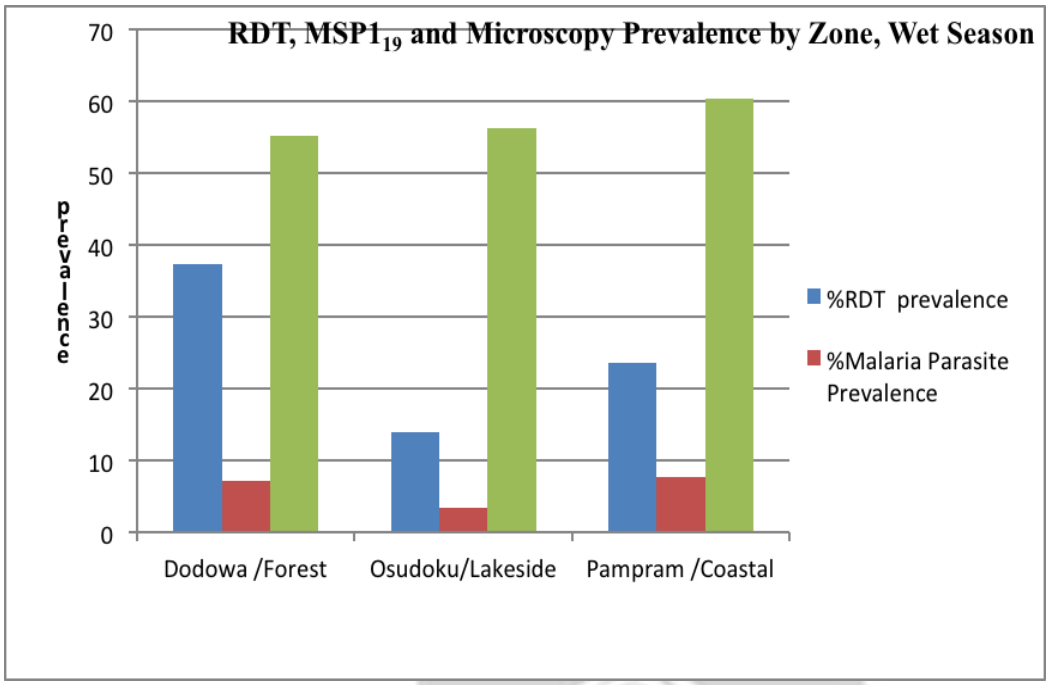


RDT positive, MSP-1_{19} positive and Malaria Parasite Prevalence by age followed the same trend by age. The highest burden of asymptomatic carriage of malaria parasites was borne by the 11-20 year old age group.

MSP-1_{19} positive prevalence estimates all malaria infections including sub microscopic infections, showing clear trends of parasite carriage burden by age. RDT rates, though lower than MSP-1_{19} positive prevalence, followed the same trend though not as marked as the MSP-1_{19} positive and parasite prevalence trend (Figure 4.21).

The wet season prevalence results also clearly shows RDT rates as a good predictor of trends of malaria burden, enhancing underlying inequalities in malaria infection distribution by age compared to microscopy results, especially as malaria transmission declines (Figure 4.22).

Figure 4.22: RDT, MSP-1$_{19}$ and Microscopy Prevalence by Zone, Wet Season



There was no significant variation in MSP-1$_{19}$, but for RDT positive prevalence, Dodowa zone had the highest of about 37% followed by Prampram and Osudoku with the least of 14%. The most significant variation was in malaria parasite prevalence, Dodowa and Prampram had almost 7% with Osudoku having half the risk of infection.

The crude incidence rates by RDT followed the same pattern as that estimated by microscopy, though expectedly, it generated a higher estimate of malaria incidence compared to microscopy (The excess rates are presented in Table 4.14). Overall, crude malaria incidence rates were high in the 0-9 years age group, dropping sharply and plateauing at age 20 years and above (Figure 4.23).

Figure 4.23: RDT versus Microscopy

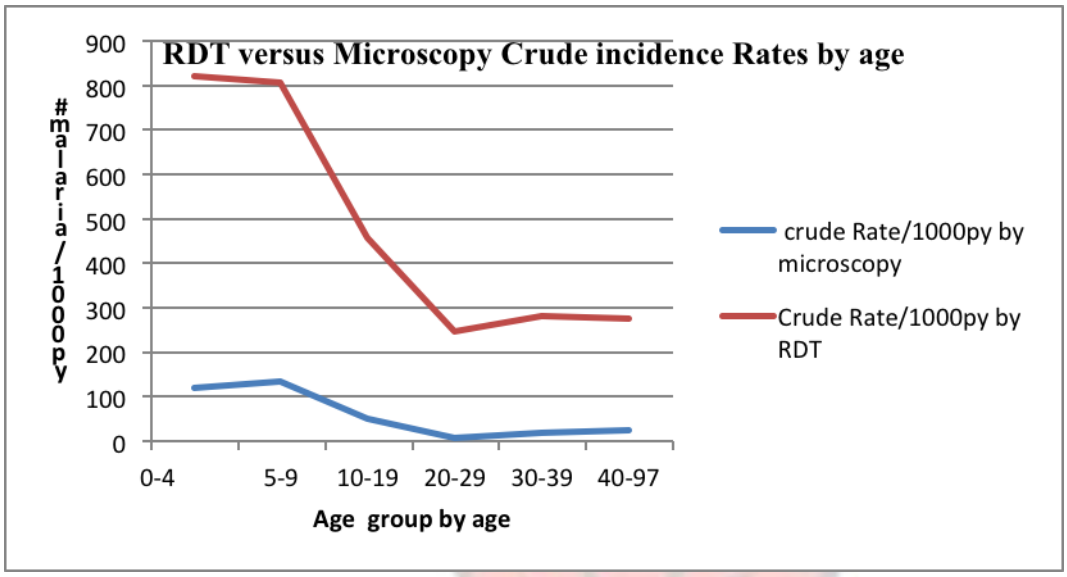
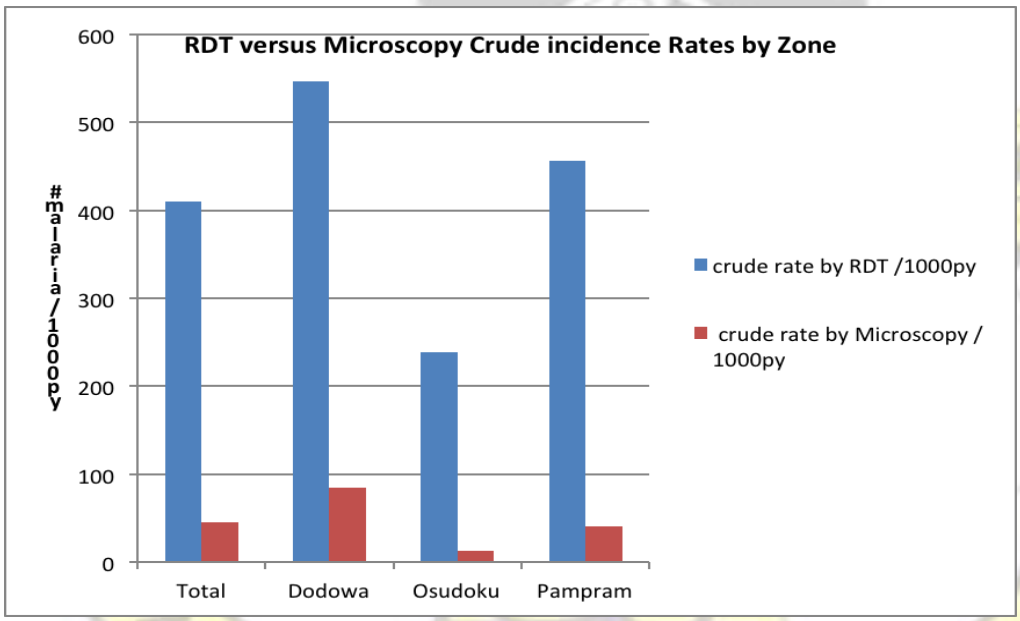


Figure 4.24: RDT versus Microscopy Crude Incidence Rates by Zone



Crude malaria incidence rates by zone showing Dodowa with the highest rates with Osudoku having the least.

Comparison of crude malaria incidence rates by zone showed RDT estimates following the same trend as Microscopy rates. Dodowa had the highest rate with

Osudoku having the least rates (Figure 4.24).

CHAPTER 5 DISCUSSION

5.1 Malaria Prevalence

Variation in the Demographic Composition of the samples for the two surveys The sex distribution of the study population was skewed towards female (60% in the dry season and 63% in the wet season), though the Health Demographic Surveillance System data (2011) reported a district male representation of 46.4%. This is especially true because the field visits for data collection were done throughout the week and for both surveys each cluster was visited only once, and participants who were absent were replaced. Also, though community mobilization towards the survey was undertaken, those who went to work or those who worked outside the community on the day of visit were excluded by virtue of their absence on the day of survey. On weekdays in particular some particular professional/ gender groups or those who worked outside the community could have been under represented. The Dodowa Township is the most urbanized and is the District capital with the entire administrative infrastructure. It has the largest population of formal sector workers, who by virtue of work may have been excluded from participation in the survey in communities, which were visited during formal working hours. The 0-10 age group was oversampled, after the underlying natural population characteristics of the district (the population of the 0-15 age is 40.5%, Dodowa HDSS, 2011). Males formed about 40% of the sample in general; Dodowa with the highest population of formal sector workers had the least of 35%, reflecting the time of data collection in the area, which fell on working days. ITN ownership was highest with Osudoku having the highest

36% whiles both Dodowa and Prampram had 33% and 32% respectively.

5.1.1 Risk of malaria infection

Current data on malaria parasite prevalence from West Africa for comparison is scarce (Satoguina *et al*. 2009), but elsewhere in Africa, many countries have reported

substantial decreases in malaria disease burden, including South Africa, Mozambique, Swaziland, Eritrea, Ethiopia and Kenya (Murray *et al*. 2012; O'Meara *et al*. 2010; Steketee & Campbell 2010).

Available data reported from Senegal and Gambia show dramatic falls in both prevalence and incidence in malaria (Trape *et al*. 2014; Ceesay *et al*. 2010; Ceesay *et al*. 2008) and some sites in Burkina Faso have also reported modest declines (Geiger *et al*. 2013), though there has been reports of no declines in malaria burden in Nigeria, Zimbabwe and Burkina Faso (Steketee & Campbell 2010). Some sites in Uganda and Mali have also reported no decreases despite scale up of malaria interventions (Coulibaly *et al*. 2014; Proietti *et al*. 2011). Reports from some sites in the Ivory Coast also shows high point prevalence of malaria in excess of 50% in children (Knoblauch *et al*. 2014). In northern part of Ghana, malaria prevalence continues to be persistently high despite control efforts (Ghana Statistical Service 2012; Owusu-Agyei *et al*. 2009)

In the dry season of 2011, the district had a malaria parasite prevalence of 7.1% parasite prevalence (18% RDT positive) and in the wet season, a 6.8% parasite prevalence (24% RDT positive), compared to the 1994 levels (At Dodowa, the malaria parasite prevalence ranged from 42.2% in the dry season to 51.3% during the rainy season, while in Pampram the prevalence were 20% and 36.6% in the dry and wet season respectively). It showed a loss of seasonality as well as a marked reduction from the 1994 levels. The reported prevalence for the dry season is higher than the 2011 Ghana Malaria multiple indicator survey (MICs), conducted in the same period which estimated the malaria parasite prevalence in children aged 0-5 years of the Greater Accra Region, of which the district is part (but is one of the most rural) at 4.1% (10.1% RDT positive).

The risk of malaria infection was similar between the forest and coastal zones, but both zones had a significantly higher risk (excess risk of 22% in the dry season) than the lakeside zone, though the lakeside zone has large irrigated fields and is more rural.

Dodowa had a marked reduction from the 1994 levels, the prevalence of malaria (parasite rate) ranged from 7% to 7.3% from the wet to the dry, compared to 51.3% during the wet to 42.2% in the dry seasons in 1994. In Prampram the prevalence was 7.7% and 7.8% in the wet and dry seasons, compared to 36.6% and 20% for the same seasons of 1994 respectively (Afari *et al*. 1995).

The disease risk showed no significant variation between the sexes, unlike in Navrongo where males significantly had a higher risk (58%) than females (38%) largely due to oversampling of males (Koram *et al*. 2003).

The age distribution of parasite prevalence as well as RDT positive prevalence varied with the 11 to 20 year age group bearing the largest asymptomatic pool for both wet and dry seasons (11.4 and 9.4% parasite prevalence by microscopy, 38 and 18.8% RDT). The discordance levels between RDT and microscopy compares favorably with data from Gambia (Satoguina *et al*. 2009) but is in disagreement with data from Burkina Faso (Tiono *et al*. 2014), where the overall RDT positive prevalence was lower than prevalence by microscopy (24.6%RDT positive prevalence versus 31.8% prevalence by microscopy).

The burden of asymptomatic malaria infections and malaria incidence fell on those in the 11-20 and 0-10 year old age groups respectively. Classically, Malaria incidence followed an increasing trend by age, from zero years until 20 years when it plateaued (Owusu-Agyei *et al*. 2001). The wet season trend for asymptomatic infections rose from age zero to a peak above 11 years of age.. The risk ratios for the age groups was one,

for the dry season, showing no difference in infection risk in the dry season, but in the wet season, those aged >20 years old group significantly had about 35% risk of infection than those aged less than 20 years (Table 4.7). There was steady RDT positivity in the dry season and may be explained perhaps by people having better access to treatment in the dry season and hence infections were short-lived and making the period prevalence low. Malaria incidence by age also showed a clear difference in burden between the 0-20 and >20years age groups, with the 0-20 year group bearing the higher burden expectedly (Table 4.15).

5.2. Malaria Incidence Rates

The Dodowa zone malaria incidence rate by microscopy of 85/1000py is an improvement over the 1994 level of 107/1000py, as the Prampram zone, 41/1000py compared to 69/1000py in 1994. Significantly, Dodowa had a 2.3 fold increase in rate of malaria over Osudoku, with Prampram having a twofold increase in rate over the same area, which could be accounted for by the fact that Osudoku had the highest prevalence of ITN ownership and use on the night before survey.

Overall rates decreased by 40% from the 1994 levels. The Lakeside zone had the lowest malaria incidence (same trend as observed malaria prevalence) despite vast irrigated fields. The Forest zone, with the lowest verified ITN use and the 5-9 year group bore the brunt of morbidity. The overall trend of EIR by month was similar to that of incidence, and these observations are consistent with each other (figure 4.19).

5.2.1 RDT versus Microscopy Malaria Risks and Rates

All RDTs were done in the field. They were fast, easy to do and read and field workers could read results after training and did not need experts to read. For all malaria prevalence and incidence rates by zone, season or age, RDT derived rates and

prevalence followed malaria parasite rates and prevalence trends as shown by figures 4.18, 4.19, 4.20 and 4.21.

The wet season prevalence results also clearly supports the assertion that RDT rates are a good predictor of trends of malaria burden enhancing underlying inequalities in malaria infection distribution by age, especially as malaria transmission declines. This is in agreement with similar work done in DR Congo (Ilombe *et al*. 2014) and in Ghana at Asutuare in the same district (Bosomprah 2014). Comparison of crude malaria incidence rates by zone showed RDT estimates following the same trend as Microscopy rates. Dodowa had the highest rate with Osudoku having the least rates.

The sensitivity of RDTs to diagnose malaria at the population level using microscopy as the gold standard was almost the same for both seasons (76% dry season, 75% wet season), and compares with results obtained in the pre rainy season prevalence study which covered both adults and children in Burkina Faso, of 77%, but was lower than the first two prevalence studies conducted two months prior to the pre rainy season survey in the same area and same population of over 85% (Tiono *et al*. 2014). The agreement level of about 86% also compares favorably with the agreement level of almost 90% in another study conducted in Burkina Faso by (Samadoulougou *et al*. 2014). The specificity was higher in the dry season than in the wet season, 87% and 76% respectively, both higher than 50% specificity obtained in (Samadoulougou *et al*. 2014)'s study but comparable with 75-96% in another study in Burkina Faso (Tiono *et al*. 2014). This may be due to the lower EIR during the dry season, decreasing the risk of exposure to malarial antigens, or the faster clearance of malarial antigen HRP2 from the peripheral blood. The wet season positive predictive value was half that of the dry season, both at the lower range of (Tiono *et al*. 2014)'s 23-74% but far lower than

(Samadoulougou *et al*. 2014)'s 76%. This can be explained by the higher number higher RDT positive prevalence during the wet season.

MSP-1_{19} positive prevalence estimates all malaria infections including sub microscopic infections (Drakeley & Cook 2009), showing clear trends of parasite carriage burden by age. RDT rates, though lower than MSP-1_{19} positive prevalence, followed the same trend though not as marked as the MSP-1_{19} positive and parasite prevalence trend.

The wet season followed for the most part the dry season characteristics. Positive response to ITN ownership was lower, 25% compared to 31% for the dry season, which compares favorably with HDSS data for the same period of 32.2% (Dodowa Health Research Center 2011). The Wet Season survey was the first to be conducted, and the low positive Response to ITN ownership may be due to the fact that participants may have answered in the negative in anticipation of being supplied free bed nets. RDT positive prevalence differed significantly by zone; Dodowa zone had the highest risk (37.2%) with Osudoku zone having less than half the risk as Dodowa zone (13.9%) (Table 4.1). The Wet Season Measured Fever prevalence on the day of study was more than double (2.3%) the dry season risk (0.7%) throughout the district, this time Dodowa zone had the highest risk of 2.9%. Parasite prevalence by microscopy also varied significantly between Dodowa and Prampram zones on one hand (7%, and 7.7% respectively) and Osudoku zone with about half the risk (3.4%).

5.3. Entomological Inoculation Rates

The dominance of *An. gambiae* as the major vector is in agreement with the finding by (Appawu *et al*. 2001; Afari *et al*. 1995). Anopheles were most abundant in May following the early rains in April, and decreased to almost zero levels in March, reflecting the rainfall patterns.

The Lakeside zone recorded the greatest abundance of Anopheles and the highest EIR of all with the Coastal zone having the least (a third of the Lakeside) which compares favorably with earlier reports from the study area (Appawu *et al*. 2004; Appawu *et al*. 2001). The annual EIR for the district followed three peaks, the highest in May, followed by July and December.

Entomological inoculation rates and malaria incidence rates variation by month through the period of study for the district followed expected trends. Peaks in incidence rates lagged behind Entomological inoculation rates by about a month. Also, whiles the entomological inoculation rates showed three peaks, the incidence rate followed only two peaks, which occurred in May and August. The malaria prevalence also did not vary seasonally, showing no significant difference between the end of wet and dry season prevalence. It also did not vary with the Entomological inoculation rates.

The forest zone produced half the total anopheles catch for the whole district, 334/697, (Tables 4.16, 4.17), with only 6% testing positive for circumsporozoite protein compared with 16% in the Lakeside zone and 3% in the coastal zone.

5.4. Implications for the Malaria Control Program

Generally as malaria transmission declines, heterogeneity in transmission levels, even in small geographical areas, becomes prominent and pronounced, increasing the need to target evidence-proven combinations of specific interventions to persons, areas and seasons.

The difference in risk between the 0-20 years old group and >20 years old group was marked in the wet season, whereas there was very little risk difference between the two age groups in the dry season (Table 4.7). This shows that persons aged 0-20 years are more vulnerable during the wet season and may need interventions targeted at them

especially just before the early rains set off. Malaria incidence by age also showed a clear difference in burden between the 0-20 and >20years age groups, with the 0-20 year group bearing the higher burden expectedly (Table 4.15).

Anopheles were most abundant in May following the early rains in April, and decreased to almost zero levels in March, reflecting the rainfall patterns throughout the district. District wide ITN distribution may have to be done before the rains start. The Osudoku Zone with the largest open water bodies had the most abundant anopheles mosquitoes and highest EIR, and may benefit from targeted IRS especially in May, just after the early rains, before the heavy rains start in June through July.

5.5. Implications for further Malaria Research

Asymptomatic parasite carriage was highest in the 9-20 years age group (table 7, 9). Further research is needed to understand further the risk of asymptomatic carriage and test the combination of interventions which best addresses the burden of asymptomatic disease in school age children. (Some interventions to be considered are Targeted Mass drug administration combined with ITNs or Targeted Testing and Treatment complemented with ITNs distribution) Fortunately, within the District about 85% of children aged between 10 and 19 years are currently enrolled in schools (Dodowa Health Reserch Center 2015), making them accessible.

The results of this study have provided current comprehensive data on malaria epidemiology, but will need updating with time without going through the expensive, long and laborious methodology used here. There is therefore the need to test and calibrate methods of using easy access populations such as participants at immunization clinics, antenatal clinic and data from multiple malaria studies (Giorgi *et al*. 2013) to estimate district malaria transmission levels quickly.

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CHAPTER 6 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusion

The incidence of malaria, in the Dangme West district, along the eastern coast of Ghana, between April 2011 and March 2012 was estimated at 45/1000person years. It varied significantly between the three ecological zones in the district. The Dodowa (Forest) zone had the highest incidence of 85/1000py, followed by Prampram (Coastal) with 41/1000py with Osudoku (Lakeside) having the lowest of 13/1000py.

The incidence rate also varied significantly between the age groupings, the 0-4 years had an estimated incidence of 119/1000py, 5-9 year had 136/1000py, 10-19 year had 50/1000py, absence of a ceiling in a room was significantly associated with an excess risk of 15%.

The prevalence of malaria parasite in the district for the same period showed no seasonality. The end of wet season (August 2011) prevalence was 6.8% whiles the end of dry season (March 2012) prevalence was 7.1%.

Malaria parasite prevalence for the Dangme West District did not differ significantly between the end of rainy season in (August) 2011 and end of dry season (March) 2012. Though the malaria parasite prevalence also varied between the zones, the difference was not significant for the dry season; Dodowa had a prevalence of 7.3%, Prampram 7.8% and Osudoku 5.6%. During the wet season, the prevalence differed significantly, statistically, between the Dodowa (7%) and Prampram (7.7) zones and Osudoku (3.4%).

The prevalence also varied significantly between the age group for both (dry/ wet) seasons, 0 to 10 year group had 6.7%/9.6%, 11 to 20 years had 9.4%/11.4%, 21 to 30 years had 6.6%/3.4%, 31 to 40 years had 6.3%/3.7% and the 41plus years had 6.9%/3.6% respectively.

HRP-2 Rapid Diagnostic Tests were used for both malaria prevalence surveys and incidence study. There were all done and read in the field. In both prevalence and incidence studies, RDT and MSP-1_{19} overestimated malaria risk and rates compared with Microscopy, but followed the same trends in risk and rate ratios prediction by seasonality and age.

The estimated Entomological Inoculation rate for the district during the period was 81 infective bites per year. The most intense transmission occurred in April, with an estimated 1 infective bite per night. Osudoku had the highest EIR of 100 infective bites per person per year, followed by Dodowa with 81, whiles Prampram had the least with 30 infective bites per person per year.

The main vector species was *An. gambiae* s.l, which constituted 95%, with *An. funestus* Giles forming the rest.

This study has showed the shifting of the burden of malaria asymptomatic carriage and disease onto school going children (9-20 years) especially in the wet season, who currently are not being targeted with specific interventions. There is a need to find the best combination of interventions to control malaria in this age group, especially in the wet season when they become most vulnerable. There is also a question of which is the best combination of interventions for the different transmission areas, seasons and age groups in the same district as transmission levels drop even further. Finally, this thesis raises the question of how best to update our understanding of the epidemiology of malaria, quickly and affordably, to enable further improvements in malaria control.

6.2 Recommendations

6.2.1 Recommendations for Malaria Control Programing

The NMCP needs to develop innovative interventions, such as school based ITN distribution with test and treat program that target the 11-20 year age group to reduce asymptomatic malaria parasite carriage.

It is recommended for the District Health Management team and NMCP to

- Improve access to test and treat facilities especially at Osudoku. Clinicians need to be trained on management of febrile illnesses so as to comply with test and treat guidelines
- Improve access to LLINs by community members especially before the start of the rainy season to reduce malaria risk

The DHMT and District partners are also encouraged;

- to strengthen routine malaria data collection and its use to support control efforts and supervise and support clinical staff to adhere to test and treat guidelines
- to continue community and patient education to support test and treat guidelines.
- educate community members on continuing malaria risk, especially before the rainy season to improve use of LLINs.
- mobilize support from district partners,(the District Assembly, decentralized agencies, departments, Non Governmental Organizations, Community leadership and Organizations) to support malaria control efforts in the district

It is recommended that Community Members;

- should continue the use of LLINs and the other malaria preventive measures available in the district
- report all febrile illnesses to the health facilities, pharmacies and chemical shops with ACTs and RDT testing facilities
- always test for malaria before taking ACTs

6.2.2 Recommendation for Further Research

I recommend the following research topics, which arose from this work.

- The risk of asymptomatic malaria parasite carriage and test the combination of interventions, which best address this.
- Research to test and calibrate methods of using easy access populations such as participants at immunization, antenatal clinics, routine malaria data from facilities and data from other malaria studies to update malaria transmission intensity data.
- Insecticide resistance monitoring at Osudoku, are the agricultural pesticides in use at the flooded rice fields having any effect on *Anopheles* population and malaria transmission?

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APPENDICES

Appendix 1: Ethical Approval, Committee on Human Research Publication and Ethics

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY
COLLEGE OF HEALTH SCIENCES
SCHOOL OF MEDICAL SCIENCES / KOMFO ANOKYE TEACHING HOSPITAL
COMMITTEE ON HUMAN RESEARCH PUBLICATION AND ETHICS

Our Ref: CHRPE/189/10

February 23, 2011

Dr. Alberta Amu Quartey
Department of Community Health
KNUST - Kumasi

Dear Madam,

LETTER OF APPROVAL
Protocol Title: ***"Estimation of Malaria Transmission Intensity in Southern Ghana Using RDT Derived Seroprevalence Rates"***

Proposed Sites: Dangme West District, Greater Accra
Sponsor: Malaria Capacity Development Consortium (MCDC)

Your submission to the Committee on Human Research Publication and Ethics on the above named protocol refers.

The Committee has considered the ethical merit of your submission and approved the protocol. The approval is for a fixed period of one year, renewable annually thereafter. The committee may however, suspend or withdraw ethical approval at anytime if your study is found to contravene the approved protocol.

Data gathered for the study should be used for the approved purposes only. Permission should be sought from the committee if any amendment to the protocol or use, other than submitted, is made of your research data.

The Committee should be notified of the actual start date of the project and would expect a report on your study, annually or at close of the project, whichever one comes first. It should also be informed of any publication arising from the study.

Thank you Madam, for your application.

Yours faithfully,

Osomfuor Prof. Sir J. W. Acheampong MD, FWACP
Chairman

cc: File

Appendix

2: Ethical Clearance

GHANA HEALTH SERVICE ETHICAL REVIEW COMMITTEE

In case of reply the number and date of this Letter should be quoted.

My Ref. :GHS-ERC: 3
Your Ref. No.

Research & Development Division
Ghana Health Service
P. O. Box MB 190
Accra

Tel: +233-0302-681109
Fax + 233-0302-685424
Email: Hannah.Frimpong@ghsmail.org

July 29 , **2011**

ALBERTA AMU QUARTEY, Principal Investigator

ETHICAL CLEARANCE - ID NO: GHS-ERC: 03/5/11

The Ghana Health Service Ethics Review Committee has reviewed and given approval for the implementation of your Study Protocol titled:

"Estimation of Malaria Transmission Intensity in Southern Ghana using RDT Derived Sero-Prevalence Rates"

This approval requires that you submit periodic review of the protocol to the Committee and a final full review to the Ethical Review Committee (ERC) on completion of the study. The ERC may observe or cause to be observed procedures and records of the study during and after implementation.

Please note that any modification of the project must be submitted to the ERC for review and approval before its implementation.

You are also required to report all serious adverse events related to this study to the ERC within seven days verbally and fourteen days in writing.

You are requested to submit a final report on the study to assure the ERC that the project was implemented as per approved protocol. You are also to inform the ERC and your mother organization before any publication of the research findings.

Please always quote the protocol identification number in all future correspondence in relation to this protocol

SIGNED.....

PROFESSOR FRED BINKA
(GHS-ERC CHAIRMAN)

Cc: The Director, Research & Development Division, Ghana Health Service, Accra

Appendix

3: Permission Granted for the conduct of Study

In case of reply the number and date of this Letter should be quoted.

My Ref: ADM/DHRC/11/06/21

Your Ref. No.SPH/VS/FT/131210

Dodowa Health Research Centre
Ghana Health Service
P. O. Box DD1
Dodowa

Tel: +233-22-25223/ 22-252238

Email: Margaret.gyapong@4u.com.gh

16 May 2011

Dr. Alberta Amu
Dangme West District Hospital
Dodowa

Dear Madam,

RE: PERMISSION TO CONDUCT RESEACRH IN THE DANGME WEST DISTRICT

With reference to your letter dated 10th May 2011 on the above subject matter, we wish to inform you that, you have been granted permission to conduct a field research titled "The estimation of malaria transmission intensity in rural southern Ghana using RDT derived sero-prevalence rates" in the Dangme West District.

Yours Sincerely,

for Dr. Margaret Gyapong
Director, DHRC

Appendix

4: Assent form, Malaria Incidence Study

(For participants 10-17 years, after parental proxy consent has been given)

Project: Estimation of malaria transmission intensity in Southern Ghana using RDT derived sero-prevalence rates.

Malaria Cohort Study

Project Area: Dangme West District.

Principal Investigator: Dr Alberta Amu Quartey (0244274807)

We are from the Dodowa Health Research Center, conducting a study to find out who, how many and where in the Dangme West District is affected most by malaria, and as part of the study, we are interviewing persons who have been selected using lottery throughout the district.

You have been selected as part 715 persons selected in this zone of the Dangme West District and will be visited at home once every month. During the visit you will be asked if you have had fever during the past month. If your answer is yes, a drop of blood will be taken from your finger to check if there are malaria parasites in your blood right in your house. If your test is positive, and you have not taken any antimalarial drug, you will be given free anti-malarial drug (Artesunate Amodiaquine). If you are very ill, you will be referred to a health facility at your own cost. Participation in this study is entirely voluntary.

I wish to invite you personally to participate in this study. The information that you provide will be written on the forms, which will be kept in locked cabinets, only to be accessed by persons authorized to do so and will only be used to understand who and where malaria is affecting us the most in this district.

Your information will be used together with the information provided by other persons who also accept to participate.

The risks involved are a little pain when the blood drop is being taken, but you benefit by knowing whether you have malaria parasites in your blood or not.

Your name will be written on our forms so we can contact you in case we want further clarification on the information you gave. However, when the information is put together with those from other people, nobody else will have access to your name or what you said.

We have asked your parents and they have agreed to let you participate.

Appendix

You are encouraged to ask questions about this study at any stage, from the field workers or me.

If you need more information, you can ask our coordinator, Dr Margaret Gyapong or write to: The Director, Dodowa Health Research Centre, P. O. Box 1, Dodowa

ASSENT FORM

Statement of person obtaining Assent:

I have fully explained this research to _____ and have given sufficient information, including that about risks and benefits, to enable the prospective participant make an informed decision to or not to participate.

DATE: _____ NAME: _____

Statement of person giving assent:

I have read the information on this study/research or have had it translated into a language I understand. I have also talked it over with the interviewer to my satisfaction.

I understand that my participation is voluntary (not compulsory).

I know enough about the purpose, methods, risks and benefits of the research study to decide that I want to take part in it.

I understand that I may freely stop being part of this study at any time without having to explain myself.

I have received a copy of this information leaflet and consent form to keep for myself.

Name_____

DATE: _____ SIGNATURE/THUMB PRINT: _____

Appendix 5: Assent form, Malaria Prevalence Study

(For participants 10-17 years, after parental proxy consent has been given)

Project: Estimation of malaria transmission intensity in Southern Ghana using RDT derived sero-prevalence rates.

Malaria Prevalence Study

Project Area: Dangme West District.

Principal Investigator: Dr Alberta Amu Quartey (0244274807)

We are from the Dodowa Health Research Center, conducting a study to find out who, how many and where in the Dangme West District is affected most by malaria, and as part of the study, we are screening persons who have been selected using lottery throughout the district.

You have been selected as part 1155 persons selected in this zone of the Dangme West District and will be screened for malaria. You will be asked questions about whether you use insecticide treated bed net, fever in the last two weeks, use of antimalarial drugs and the use of antenatal services if you are pregnant. After this, we will take your temperature and a drop of blood from your fingertiP. If your test is positive, and you have not taken any antimalarial drug, you will be given free antimalarial drug (Artesunate Amodiaquine). If you are very ill, you will be referred to a health facility at your own cost. Participation in this study is entirely voluntary.

I wish to invite you personally to participate in this study. The information that you provide will be written on the forms, which will be kept in locked cabinets, only to be accessed by persons authorized to do so and will only be used to understand who and where malaria is affecting us the most in this district.

Your information will be used together with the information provided by other persons who also accept to participate.

The risks involved are a little pain when the blood drop is being taken, but you benefit by knowing whether you have malaria parasites in your blood or not.

Your name will be written on our forms so we can contact you in case we want further clarification on the information you gave. However, when the information is put together with those from other people, nobody else will have access to your name or what you said

We have asked your parents and they have agreed to let you participate.
You are encouraged to ask questions about this study at any stage, from the field workers or me.

If you need more information, you can ask our coordinator, Dr Margaret Gyapong or write to: The Director, Dodowa Health Research Centre, P. O. Box 1, Dodowa

ASSENT FORM

Statement of person obtaining Assent:

I have fully explained this research to _____ and have given sufficient information, including that about risks and benefits, to enable the prospective participant make an informed decision to or not to participate.

DATE: _____ NAME: _____

Statement of person giving assent:

I have read the information on this study/research or have had it translated into a language I understand. I have also talked it over with the interviewer to my satisfaction.

I understand that my participation is voluntary (not compulsory).

I know enough about the purpose, methods, risks and benefits of the research study to decide that I want to take part in it.

I understand that I may freely stop being part of this study at any time without having to explain myself.

I have received a copy of this information leaflet and consent form to keep for myself.

Name_____

DATE: _____ SIGNATURE/THUMB PRINT: _____

Appendix 6: Consent form, Malaria Incidence Study

Project: Estimation of malaria transmission intensity in rural Ghana using RDT derived sero-prevalence rates. Malaria Follow Up Study

Project Area: Dangme West District

Principal Investigator: Dr Alberta Amu Quartey (0244274807)

My name is Dr Alberta Amu Quartey, from the Dodowa Health Research Centre. We are conducting a study to find out who, how many and where in the Dangme West District is affected most by malaria, and as part of the study, we are interviewing persons who have been selected by lottery throughout the district. You have been selected to participate in this study.

If you agree to participate (or your ward) in this study, will be visited at home once every month. You will be asked some questions about malaria, including a question on if you have had fever in the past 2 weeks or month. If your answer to the fever question is yes, a drop of blood will be taken from your fingertip to check if there are malaria

parasites in the blood right in your house. If malaria parasites are found in your blood, you will be given free anti-malarial drug or referred to a health facility at your own cost if you are very ill or thought to be suffering from other illness. You may refuse to answer any questions you are uncomfortable with.

I wish to invite you personally to participate in this study. Participation is entirely voluntary and withdrawal will not affect any benefit you are entitled to within this district. It is a minimum risk study.

The information that you provide will be written on the forms, which will be kept in locked cabinets, only to be accessed by persons authorized to do so and will only be used to understand who and where malaria is affecting us the most in this district. Your information will be used together with the information provided by other persons who also accept to participate.

Your name will be written on our forms so we can contact you in case we want further clarification on the information you gave. However, when the information is put together with those from other people, nobody else will have access to your name or what you said.
The information from this study will be shared with the community, the Dangme West Health Management Team, the District Assembly and the National Malaria Control Program, to help improve the control of malaria in this district.

You are encouraged to ask questions about this study at any stage, from me, the field workers or from the Directors at Dodowa.

If you need more information, you can ask our coordinator, Dr Margaret Gyapong or Dr Evelyn Ansah at the Dodowa Health Research Office or District Health Administration at Dodowa

You can also write to them:

The Director,
Dodowa Health Research Centre,
P. O. Box 1,
Dodowa

District Director of Health Services, P.O.
Box 1.
Dodowa

If you want them to contact you, please give your contact to the interviewer, for the coordinators to contact you.

If you are willing to participate, please sign below to indicate this fact.

Signature----------

Or

Thumbprint……..... (Please indicate RTP or LTP).

Witness……

Date……

Thank you

Appendix 7: Consent form for room owner, Entomology Study

Project: Estimation of malaria transmission intensity in rural Ghana using RDT derived sero-prevalence rates. Entomological study

Principal Investigator: Dr Alberta Amu Quartey (0244274807)

My name is Dr Alberta Amu Quartey, from the Dodowa Health Research Centre and carrying out a study on malaria in the Dangme West district. Mosquitoes carry the germ that causes malaria, so the control of mosquitoes is important in the control of malaria. Within this district, control of mosquitoes has been done using insecticide treated bed nets (ITN) and recently indoor residual spraying. Many programs have been done and still ongoing to reduce the effect of malaria in the district. In order for us to know how well these programs are working, we need to know the intensity of malaria in the district. We measure this by measuring the level of transmission of malaria in the district.

In order for to estimate the current status of malaria transmission in the district, we need to catch mosquitoes during the day and at night from randomly selected houses in the Dangme West district. This will last for one year but we will work in your compound only for one night and the morning of the next day. Two fieldworkers will sit in one room that you (Head of household) will give to the fieldworkers, the other two in the compound of your house, but not in a room to collect mosquitoes. The mosquitoes will be collected from 6.00pm to 6.00am the next morning. The workers will also fix a cage in some windows in your house to collect any mosquitoes that will be leaving the rooms.

The next morning they will spray some rooms in the house with mosquito spray (Raid which contains 0.15%Tetramethrin, 0.25% Allethrin, and 0.015% Delmethrin, produced by Johnson Wax European BV Holland) to collect the mosquitoes that will be knocked down.

Your house is one of the houses selected from the district in a manner that house numbers are drawn randomly like in a lottery.

I am personally inviting you to be part of the study, by giving us your room for mosquito collection.

Mosquitoes collected from your house will be sent to the laboratory at the Dodowa Health Research. They will then be sorted into the various species and counted. Some of the mosquitoes caught will be preserved in dry form and used to find out if they carry malaria parasites.

I do not know of any risks associated with this study in terms of members of your compound or those who will sleep in the room later. There will be some inconveniences when strangers like our fieldworkers come into your room to collect mosquitoes. The workers are well trained to show courtesy and respect all the norms and culture of your household as they carry out their work.

There will however be some benefits to those who will sleep in the room after mosquito collection, as most of the mosquitoes would have been collected. The mosquito spray will reduce the number of mosquitoes in the particular room and the compound as a whole.

Data collected from your room will not be given to any third party. Reports of results of the study will not be linked to any room but will be reported as district-wide information. We will collect data on forms specifically for this study. We will keep the forms for your house and all other houses in files that will be in locked bins.

Allowing us to collect mosquitoes from your room is not by force. You may refuse if you do not want to be part of the study. If you do not have a place to sleep to allow us use your room for one night, we will move to another house. You may withdraw at any time during the study if you join but decide to change your mind later.

You are encouraged to ask questions about this study at any stage, from me, the field workers or from the Directors at Dodowa.

If you need more information, you can ask our coordinator, Dr Margaret Gyapong or Dr Evelyn Ansah at the Dodowa Health Research Office or District Health Administration at Dodowa

You can also write to them:

The Director,
Dodowa Health Research Centre,
P. O. Box 1,

Dodowa

District Director of Health Services, P.O.
Box 1.
Dodowa

Declaration

I have read the information sheet concerning the study/or have had the information sheet concerning this study translated to me in my language or English. I fully understand what is required of me if mosquitoes are going to be collected from my room and that the study will allow for the estimation of malaria transmission in my area.

I agree to participate in this study

Name.....
Signature.....

Or thumbprint (please indicate- right or left)

Date.....

Witness.....

Appendix 8: Consent form for field worker, Entomology Study

Project: Estimation of malaria transmission intensity in rural Ghana using RDT derived sero-prevalence rates. Entomological study

Principal Investigator: Dr Alberta Amu Quartey (0244274807)

My name is Dr Alberta Amu Quartey from the Dodowa Health Research Centre and carrying out a study on malaria in the Dangme West district. Mosquitoes carry the germ that causes malaria. Malaria is a major health problem in Ghana and in the Dangme West District killing many people especially children and pregnant women every year. Control of mosquitoes is important in the control of malaria. Malaria control in this district has been through the use of insecticide treated nets (ITNs), treatment of malaria illness with effective drugs and prevention of malaria in pregnancy by taking Intermittent Preventive Treatment (IPTp). Recently indoor residual spraying (IRS) has been added to the control measures.

Continuous measurement of the intensity of malaria parasite transmission is very useful in the assessment of malaria control efforts.

Malaria transmission is influenced by several factors including the vector mosquito density, vector species, feeding preferences, resting behavior and vector efficiency, among others.

In order for us to estimate the current status of malaria transmission, we need to catch mosquitoes during the day and night from randomly selected houses in the Dangme West district. This work will last for one year but you will work in a house for only one night and the morning of the next day. During the night, four of you fieldworkers will collect some mosquitoes from one house using the Landing catches method involving exposing your lower limbs as baits. Two will sit in the room to do indoor collection and the other two in the compound to do outdoor collection. The mosquitoes will be collected from 6.00pm to 6.00am the next morning. You will also do exit trap and spray catches.

You will bring all the mosquitoes caught to the laboratory in the Dangme West District Hospital where they will be killed using chloroform. The mosquitoes will then be sorted into the various species and counted. Some of the mosquitoes caught will be preserved in dry form and used to find out if they carry malaria parasites.

The aim of the study is to measure malaria transmission. To achieve this objective, there will be the need to collect adult mosquitoes off the lower limbs of mosquito collectors (fieldworkers) to assess their biting rates, biting patterns and behavior, infectivity rates etc. This procedure carries with it a little risk of infection through human error and you may become infected. You will therefore be given adequate training to reduce the

tendency of committing errors and therefore exposing yourself to infection. Any of you who show symptoms of any of the endemic diseases in the area will be sent to one of our qualified physicians for proper diagnosis and advice. Where the diagnosis indicates malaria, you will be treated with reference to the guidelines of the Ghana Health Service. Any infected and treated fieldworker will be re-checked in all cases to ensure that he is fully cured. Since mosquitoes also transmit yellow fever, I will ensure that you have taken the yellow fever vaccine by asking you to bring your yellow card for inspection. If you have not taken the yellow fever vaccination within the last 10 years, you will have to take it before you are allowed to work on this project.

Data collected from you will not be given to any third party. We will keep the forms containing your personal data in files in locked cabinets.

There is no special compensation for working on this project. You will be paid on this project as any other fieldworker of the Dodowa Health Research Centre.

Working on this project is not obligatory. Apart from ensuring that you have the yellow fever vaccination, you will be treated as any other staff of Dodowa Health Research Centre, and you will have to abide by the rules and regulations of the center regarding resignations.

You are encouraged to ask questions about this study at any stage, from me, the field workers or from the Directors at Dodowa

If you need more information, you can ask our coordinator, Dr Margaret Gyapong (0244209971) or Dr Evelyn Ansah (0244869700) at the Dodowa Health Research Office or District Health Administration at Dodowa.

You can also write to them:

The Director,
Dodowa Health Research Centre,
P. O. Box 1,
Dodowa

District Director of Health Services, P.O.
Box 1.
Dodowa

FIELDWORKER DECLARATION

I have read the information sheet concerning this study and my role as a fieldworker on the study. I understand and have also received answers to all my concerns. I fully understand what is required of me if I agree to work as a fieldworker responsible for

mosquito sample collection and that the study will allow for the estimation of malaria transmission.

I agree to work on this study.

Name of Field Worker.....
Signature.....

Date.....

Witness.....

Appendix 9: Participant Information Leaflet

Project: Estimation of malaria transmission intensity in Southern Ghana using RDT derived sero-prevalence rates.

Project Area: Dangme West District.

Principal Investigator:

Dr Alberta Amu Quartey, Kwame Nkrumah University of Science and Technology and Dangme West District Hospital (0244274807)

Supervisor:

Prof Tsiri Agbenyega, Kwame Nkrumah University of Science and Technology

BACKGROUND

Globally, 247 million cases of clinical malaria occurred in 2006, which resulted in 881000 deaths, most of which occurred in sub-Saharan Africa and in children under five years. Ghana had an estimated 7.2 million cases in 2006, contributing 3% of the total African malaria cases in that year. There has been no evidence of malaria case reduction between 2001 and 2007 and malaria deaths reportedly increased in 2007. Malaria transmission intensity is a major determinant of the disease burden, increasing levels corresponding to increasing incidence of severe disease. Since 1999, strengthened global and national efforts have been put into preventing and controlling malaria. These efforts need to be monitored and evaluated over time to assess impact and to respond to gaps in these efforts. Measuring Malaria transmission over time has been used for this purpose. Entomological studies have been the gold standard but the logistical and financial cost of this can be very taxing.

Studies have proposed and validated the relative ease and accuracy of using parasite and serological surveys to estimate the force of malaria transmission. Rapid Diagnostic Tests (RDTs) for malaria have traditionally been used in malaria diagnosis at health facilities but not within the community, but has a lot of potential to be used for malaria testing as well as a source of antibodies for measuring malaria transmission levels instead of the traditional entomological methods at the community level.

STUDY PURPOSE

This study is to explore and validate the use of RDTs as a Simple, rapid and less expensive tool for malaria transmission measurement, critically needed for monitoring trends over time to inform interventions targeted at malaria control and prevention

The study has 3 components:

Prevalence study:

3456 Participants of all ages and sexes will be selected using lottery from each of the three ecological zones in the Dangme West District. These zones are Dodowa, Osudoku and Ningo/Prampram sub-districts. This survey will be done twice within a year, timed to coincide with the end of wet season (August 2011) and the end of the dry season (March 2012)

Each participant will be interviewed using a questionnaire after a written informed consent has been sought. The questionnaire will cover name, age, house number, gender, use of bed nets and anti-malarial drug use, history of fever in the last 2 weeks and actions taken if any. Axillary temperature of participants will be taken with electronic thermometer. A finger or heel prick blood sample malaria blood tests, and hemoglobin estimation (blood level) will be done for all participants. Participants who are pregnant at the time of survey with any gestational age or delivered in the last 6 weeks or less will be interviewed with a questionnaire which will seek information on age, name, obstetric data, use of antenatal services, and history of any ill health since the last two weeks, IPTp, anti-malarial and bed net use will be sought. ITN use will then be verified. Participants found to be RDT positive will be treated appropriately or referred to a nearby health facility.

Cohort Study:

2145 participants of all ages, drawn by lottery, from each zone in the district will be visited once a month for a year from June 2011, and a history of fever within the past week elicited. Those who respond yes will be interviewed with a questionnaire which will seek to find what has been done, antimalarial drugs taken and treated bed net use. After this, a finger/ heel prick blood sample will be taken for RDT; thick and thin blood film preparation. Those who are found to be positive for malaria parasites and have not had any treatment will then be treated with anti-malarial or referred to the nearest health facility.

Entomology Study:

This part of the study is to find out how many mosquitoes are biting and transmitting malaria at the community level. Lottery will be used to select and code 4 houses for mosquito collection per zone per month during the cohort study period by: Human Landing Collections (HLC):

Four trained and consented fieldworkers will collect mosquitoes from four houses per zone per month. Two will sit indoors and two outdoors for 50 minutes of each of the hours between 1800hrs and 0600hrs to collect mosquitoes which will land on the exposed legs using flashlights and glass tubes. The two teams of collectors will rotate between indoor and outdoors hourly after taking ten minutes break. Field workers will

be provided free and rapid treatment for malaria when needed. Outdoor Collection was done between 0900-1100 hours by collecting resting adult anopheles mosquitoes from outdoor shelters.

Pyrethrum Spray Collections

During the hours of 0600hrs and 0800hrs, floors and other surfaces in rooms of the selected houses will be completely covered with white sheets and sprayed with mosquito spray. 10 minutes after spraying, the sheets will be lifted outside and knocked down mosquitoes picked uP.

ETHICS

Ethical clearance has been sought from the Kwame Nkrumah University of Science and Technology and the Ghana Health Service Ethical Review board to do this study. Community mobilization and engagement will be done prior to and throughout the study period. Written Informed Consent will be sought from participants and caregivers (in case of children).

RISKS AND BENEFITS

There are no known risks associated with the study in terms of individuals and communities who participate. There will be inconveniences with the time spent to answer questions and participate in the medical screening, but those who participate will know their blood hemoglobin level and whether they have malaria infection or not. Taking blood from the fingertips causes pain and discomfort. Some participants will also benefit from free malaria treatment or referral to a health facility if a problem is found.

There will be some inconveniences when fieldworkers come into your house or room to collect mosquitoes. Field workers will be trained to show courtesy and respect for all the norms and culture of the community and individuals as they carry out their work.

There will however be some benefits to those who will sleep in the rooms after mosquito collection, as most of the mosquitoes would have been collected. The mosquito spray will reduce the number of mosquitoes in the particular room and the house as a whole.

CONFIDENTIALITY

Data collected from participants will not be given to any third party. Reports of results of the study will not be linked to any household or individual, but will be reported as district wide information. Data will be collected on forms specifically for this study. All filled forms will be filed and stored in locked cabinets.

COMPENSATION

There is no direct compensation for participating in this study

WITHDRAWAL FROM THE STUDY

Participating in this study is entirely voluntary. Participants may refuse if they do not want to be part of the study. They may withdraw at any time during the study if they decide to change your mind. Withdrawal will not affect any benefits that they are otherwise entitled to at any health facility or within the community.

FEEDBACK

There will be Dissemination durbars, where feedback of the outputs of the study will be communicated to participating communities and stakeholders.

QUESTIONS

Participants are encouraged to ask questions about this study at any stage, from me, the field workers or from research coordinators at Dodowa

For more information, contact the coordinators, Dr Margaret Gyapong or Dr Evelyn Ansah at the Dodowa Health Research Office or District Health Administration at Dodowa

Or write to them:

The Director,
Dodowa Health Research Centre,
P. O. Box 1,
Dodowa

District Director of Health Services, P.O.
Box 1.
Dodowa

Appendix 10: Consent form, Malaria Prevalence Study

Project: Estimation of malaria transmission intensity in rural Ghana using RDT derived sero-prevalence rates. Prevalence Study

Project Area: Dangme West District.

Principal Investigator: Dr Alberta Amu Quartey (0244274807)

My name is Dr Alberta Amu Quartey, from the Dodowa Health Research Centre. We are conducting a study to find out who, how many and where in the Dangme West District is affected most by malaria, and as part of the study, we are interviewing, examining and conducting malaria laboratory tests on persons who have been selected by lottery throughout the district.

You have been selected to participate in this study. It is entirely voluntary. If you agree to participate in this study, you will be interviewed, examined clinically by qualified doctors and a blood drop from your finger or heel (in babies) will be taken to check if you have malaria. If your blood tests positive to malaria parasites, you will be given free anti-malarial drug. If you are found to be very ill, you will be referred to a health facility at your own cost. You may also decide not to answer any question you are uncomfortable with.

I wish to invite you personally to participate in this study. This is a minimum risk study.

The information that you provide will be written on the forms, which will be kept in locked cabinets, only to be accessed by persons authorized to do so and will only be used to understand who and where malaria is affecting us the most in this district.

Your information will be used together with the information provided by other persons who also accept to participate in this study

Your name will be written on our forms so we can contact you in case we want further clarification on the information you gave. However, when the information is put together with those from other people, nobody else will have access to your name or what you said.

The information when from this study will be shared with the community, the Dangme West Health Management Team, the District Assembly and the National Malaria Control Program, to help improve the control of malaria in this district.

If you are willing to participate, please sign below to indicate this fact.

Signature----------

Or

Thumbprint…….....

(Please indicate RTP or LTP).

Witness ……

Date……

You are encouraged to ask questions about this study at any stage, from me, the field workers or from the Directors at Dodowa

If you need more information, you can ask our coordinator, Dr Margaret Gyapong (0244209971) or Dr Evelyn Ansah (0244869700) at the Dodowa Health Research Office or District Health Administration at Dodowa You

can write to them:

The Director,
Dodowa Health Research Centre,
P. O. Box 1,
Dodowa

District Director of Health Services, P.O.
Box 1.
Dodowa

If you want them to contact you, please give your contact to the interviewer, for the coordinators to contact you.

Thank you.

Appendix 11: Malaria Incidence Study Questionnaire

MALARIA INCIDENCE STUDY
MONTHLY HOME VISIT QUESTIONNAIRE

INSTRUCTIONS TO INTERVIEWER: Introduce yourself and explain the purpose of your visit. Ask to speak to the participant or mother or to another Adult caretaker of participant. If this is not possible, arrange a time to revisit the house when participant or carer will be at home. Before interviewing the person, explain to him or her that the participation is voluntary, Seek for written informed consent. Explain to him/her that the information provided is only for research purposes only and as such will be kept confidential.

FW Code ☐ FS

Interview Date ☐☐☐☐☐☐☐☐

DINT

House Name……House ID ☐☐☐☐☐☐☐
H _ID

Visit	**1st**	**2nd**	**3rd**	**Final Visit**
Date (dd/mm/yyyy)				
Result 1. Completed 2. Not at home 3. Postponed 4 .Refused 5. Partly completed 6 .No appropriate respondent found 7. Other (specify).....				
Next Visit	_____ _____	_____ _____		Total Number Visits this week ☐☐

Time Interview Started HOURS/MINUTE ☐☐ Ended ☐☐☐☐

Duration ☐☐☐☐

SECTION 1.0: BASIC INFORMATION

Name of Participant……..… 1.2Gender: ☐ Male ☐ Female

1.3 Date of birth (dd/mm/yyyy) ☐ ☐
D_OB

1.4 Unique ID ☐☐☐☐☐☐☐☐☐☐☐
1.5 Name of Head of Household……..……..

1.6 Community…… 1.7 Sub district: Dodowa……..……1 ☐
Osudoku…….……2 ☐
Ningo-Pampram….3 ☐

1.8 Total number of persons in the household ☐☐ H_size

SECTION 2.0 INFORMANTS

		skip
2.1 Is the respondent the participant?	☐ Yes……..1 ☐ No…..….2	If Yes ↘ Move to 3.1

2.2 What is the name of the respondent ……
2.3 What is the relationship of the respondent to the participant?

☐ **Mother 1**	☐ **Father 2**	☐ **Grandmother 3**	☐ **Aunt 4**	☐ **Uncle 5**	☐ **Grandfather 6**	**Others, specify…7**

SECTION 3.0 HOUSING CONDITION

Code

3.1 What type of material is the roof of the room where sleeps made of? Straw/thatch……...1
Aluminum/zinc……..2 participant
Slate……3
Bricks…….4
Tiles……5
Concrete…….6
Others, specify……..7

3.2 Is there a ceiling in the room? Yes……1
No……..……2
Don't know……3

3.3 What type of material is the room in which participant sleeps made of? Brick……...1
Mud…….2
Wood…….3
Sand-crete…….4
Iron……..….5
Others, specify…..6

	☐ ☐ ☐ ☐ ☐	
3.4 Is there an open water within 30m radius of 3.5 Is there an animal	Yes, specify……....1 body No……..…...2 house? Yes, specify……..1	
	☐	**Code**
enclosure within 30m house?	No……..……...2 radius of	
3.6 Is there a farm within 30m radius of house of	☐ Yes, specify ……....1 No……..…..2 participant?	

SECTION 4.0 MALARIA ACTIONS

4.1 Did participant sleep in an insecticide treated bed net last night? 4.2 Did participant sleep in an insecticide treated bed net since the last visit? 4.3 Has the house in which participant lives been sprayed(indoor residual spraying) since the last visit 4.4 Has the surrounding area of the house in which participant lives been sprayed with insecticide spray since the last visit? 4.5 Has participant travelled outside the community in which he/she lives since the last visit? 4.6 If yes, where did participant travel to? 4.7 Did participant sleep under an insecticide treated bed-net during the period? 4.8 Is the participant a pregnant woman (if female and in childbearing age) 4.9 Has the participant taken IPT since the last visit? (if pregnant)? 4.10a. Has participant had fever since the last 2 weeks?	4.11 If yes, what is the body temperature of the participant? 4.12 Did participant take any medication for the fever? ☐ ☐ Yes……..……1 No……..……2 ☐ ☐ Yes……..……1 ☐ ……...……2 ☐ ☐ Yes……..……1 ☐ No……..……2 Don't know……..…3 ☐ ☐ Yes……..……1 No……..……2 ☐ Don't know……..……3 ☐ Yes……..……1 No……..……2 ☐ ☐ Within the zone…...…..1 ☐ To another zone within the district…....2 (Specify- Dodowa Ningo ☐ Prampram Osudoku)…. ☐ Outside the District…...3 Other, specify……...…4 ☐ Yes……..…..…1 ☐ No……..…...2 ☐ ☐ Yes……...……1 No……...……2	No ↘ If no Move to 4.8 ↘ If No
	☐ ☐ 176	↘

Yes……...……1
No……...……2
Don't know……...….3

Yes……...…1 If no
No ……...…2
Discontinue

Yes……...…1 If no
No……...…2 Move to 4.21

.☐ ºC

4.13 How long after onset of fever did participant start medication	☐ Less than 24 hours.….1 Between 1-2 days…..2 After 3 days……...3 ☐ Other specify…….....4 Don't knoow…….…..5 ☐ ☐ ☐	

4.14 Which anti-malarial medication did participant take?	4.16 № of days medication taken	4.17 Source of medication	4.18 Forms of medication	4.19 Frequency of medication
☐ **Artesunate-modiaquine..1** **Artesunate-umefantrine...2** **Chloroquine……...…..…..3** **Sulphadoxine-Pyremethamine……4** **Quinine……..…..5** **DHAP……...……6** **Don't know……..……7** **None of the above…...….8** ☐ ☐ ☐ ☐ ☐ ☐ ☐	☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ⇨ 7	☐ Hospital…….1 ☐ Health Center…2 Private Clinic….3 Mission Clinic…4 ☐ CHPS Compound5 ☐ Maternity Home..6 Pharmacy…….7 ☐ Chemical Shop…8 Drug Peddler…..9 Traditional Healer..10 ☐ Other, Specify….11 ☐ ☐ ☐ ☐ ☐ ☐	☐ Tablet……1 Capsule……..2 ☐ Syrup/Suspension3 Dispersible ranules……4 ☐ Injection…….5 ☐ Suppository……6 g Others, specify ……..7 ☐ ☐ ☐	☐ Once a day…1 d Twice a ay...2 ☐ Three mes a d ay…… …..3 ☐ Four t mes a day…… …..4)nce…… ..5 Other, pecify …… ☐ ..6 t d

4.15 If artesunate-amodiaquine (Refer to 4.14), which brand? ☐ **Coarsucam……1** ☐ **Don't know……2** ☐ **Other, specify……...…...3**				

4.20 Which other additional medications did the participant take?

☐ Antipyretic specify…….……1

☐ Antibiotic specify……2

☐ Multivitamin……...3

☐ Iron preparation……..…4

☐ Herbal preparation specify…….…….5

☐ Don't know…….6

☐ Other, Specify…..7 **Response Skip**

4.21 Have you visited a health facility for this fever? Yes…….....…….. 1 No …….....…….…2 If No Move to 4.23

4.22 If yes, what is the name of the facility?

Dangme West District Hospital Dodowa.....1
Grace Maternity Home……..2
Ayikuma CHPS Compound.....3
Agomeda CHPS…….……4
St Andrews Kordiabe……...5
Pampram Health Centre…….....6
Ningo Health Center…….....7
Omari Clinic…….....8
New Ningo CHPS…….....…....9
Godia Clinic……...….10
Osuwem CHPS.....11
Lekpongnour CHPS……....12
Ebenezer Clinic…….....…..13
Agottor CHPS……..…14
Duffor Health Center……..…15
Nyigbenya CHPS…….....16

	☐ Afienya Youth Clinic…..…….17 ☐ Gloria Maternity……..….18 ☐ Dawhenya CHPS……...…19 ☐ Others, specify……..……20	
4.23 Did participant do a blood test for malaria before being given any medication or not?	Yes……1 ☐ No…….……2 ☐	If No ↘ Move to 4.26
4.24 If yes, what type of test?	RDT……..1 ☐ Microscopy……..……2 ☐ Other, specify……..……3 ☐ Don't know……...….4	
4.25 Test results	☐ Positive……..…….1 ☐ Negative……..……2 ☐ Don't know……4	
4.26 How is participant now	☐ Recovered……..…….1 ☐ Has not recovered but ……..…2 ☐ Sick……....3 ☐ Don't know……...…..4	

5.0 BLOOD SAMPLE

INSTRUCTIONS TO INTERVIEWER- If participant responded yes to 4.10, explain to participant and obtain permission to take blood sample for malaria test. Explain that it is voluntary, and the results will be given to patient and used only for research purposes and will be kept confidential. Explain procedure to participant and gently take finger prick sample for RDT and blood film. Show RDT results and explain to participant.

5.1 Blood sample taken?	5.2 RDT results	5.3 Lab form filled?
☐ Yes……..…1 ☐ No, specify…….2	☐ Positive……..…1 ☐ Negative……..2 ☐ Indeterminate……3	☐ Yes……..1 ☐ No……..…2

5.4 Sample code: | | | | | |

Field supervisor checked	FSCODE	COMMENTS

Appendix 12: Malaria Prevalence Study Questionnaire malaria prevalence study questionnaire

INSTRUCTIONS TO INTERVIEWER: Introduce yourself and explain the purpose of the study. Seek written informed consent from participant or mother or to another Adult caretaker of participant. Before interviewing the person, explain to him or her that the participation is voluntary, Seek for written informed consent. He/she can refuse to answer any question and she/he can stop the interview at any time. Explain to him/her

that the information provided is only for research purposes, and will be kept confidential.

FW Code FS

Interview Date

DINT

House Name……..…House ID
H_ID

Season……

SECTION 1.0: BASIC INFORMATION

1.1 Name of Participant……

1.2 Gender: Male = M Female = F

1.3 Date of birth (dd/mm/yyyy) D_OB

1.3 Unique ID UNI_ID

1.4 Name of Head of Household……..

1.5 Community…….

1.6 Sub district, Dodowa……..……..1
Asutuare……..……..2
Ningo-Prampram……..3

1.7 Total number of persons in the household H_size

SECTION 2.0: INFORMANT INFORMATION

2.1 What is the name of the respondent if participant is a child……

2.2 What is the relationship of the respondent to the participant?

1. Mother	2. Father	3. Grandmother	4. Aunt

I_REL

5. Uncle	6. Grandfather	7. Others specify……..

SECTION 3.0 MALARIA ACTIONS

		Code	skip
3.1 did participant sleep in an insecticide treated bed net last night?	Yes…..……1 No…….……2		

3.2 Did participant sleep in an insecticide treated bed net this last week?	Yes…….....……..…1 No …….....…….2		
Has the house in which participant lives and sleep been sprayed with insecticide (indoor residual spraying)?	Yes…….....…….1 No…….....……2 Don't know…….....……3		
Has the surrounding area of the house in which participant sleeps been sprayed with insecticide?	Yes……..1 No……..……..2 Don't know……..……..66		
3.3 Has participant had fever this last Week?	Yes…….....…….1 No …….....….2		If no ↘ 4.0
3.4 Did participant take any medication for the fever?	Yes…….....….1 No…….....……2		If no ↘ 3.15
3.5 How long after onset of fever did participant start medication	Less than 24 hours……..1 Between 1-2 days……..…….2 After 3 days…….....…….3 Other specify…….....……...4 Don't knoow…….....……..88		
3.6 Which medications did participant take?	Antipyretic specify…….....……1 Antibiotic specify…….....….2 Multivitamin…….....……3 Iron preparation…….....….4 Artesunate-Amodiaquine…….....5 Artesunate Lumefantrine…….....6 Chloroquine…….....…7 Sulphadoxine-Pyremethamine….8 Quinine…….....……9 Herbal preparation specify..... 10 Other, Specify…….....….11		

How much of each medication, type, how often and for how long did participant take medication?

CHARACTERISTICS OF MEDICATIONS TAKEN	**DAYS**										**Duration**	**Code**
	1	2	3	4	5	6	7	CODE	5			
3.7 TYPE Antipyretic specify……..……1 Antibiotic specify……2 Multivitamin……3 Iron preparation……...…4 Artesunate Amodiaquine……...….5 Artesunate Lumefantrine…….…6 Chloroquine…….....…7 Sulphadoxine –Pyremethamine…..8 Quinine……..……9 Herbal preparation specify……10 Other, Specify……11								T_MED				
If Artesunate-Amodiaquine, which brand? Coarsucam……...…1 Others……..……2 Don't know……66												
3.8 SOURCE Hospital……...1 Health Center…….2 Private Clinic……...…….3 Mission Clinic……..…….4 CHPS Compound…….....5 Maternity Home……..…6 Pharmacy ……..….7								S_MED				

Chemical Shop……..….8 Drug Peddler……..……9												

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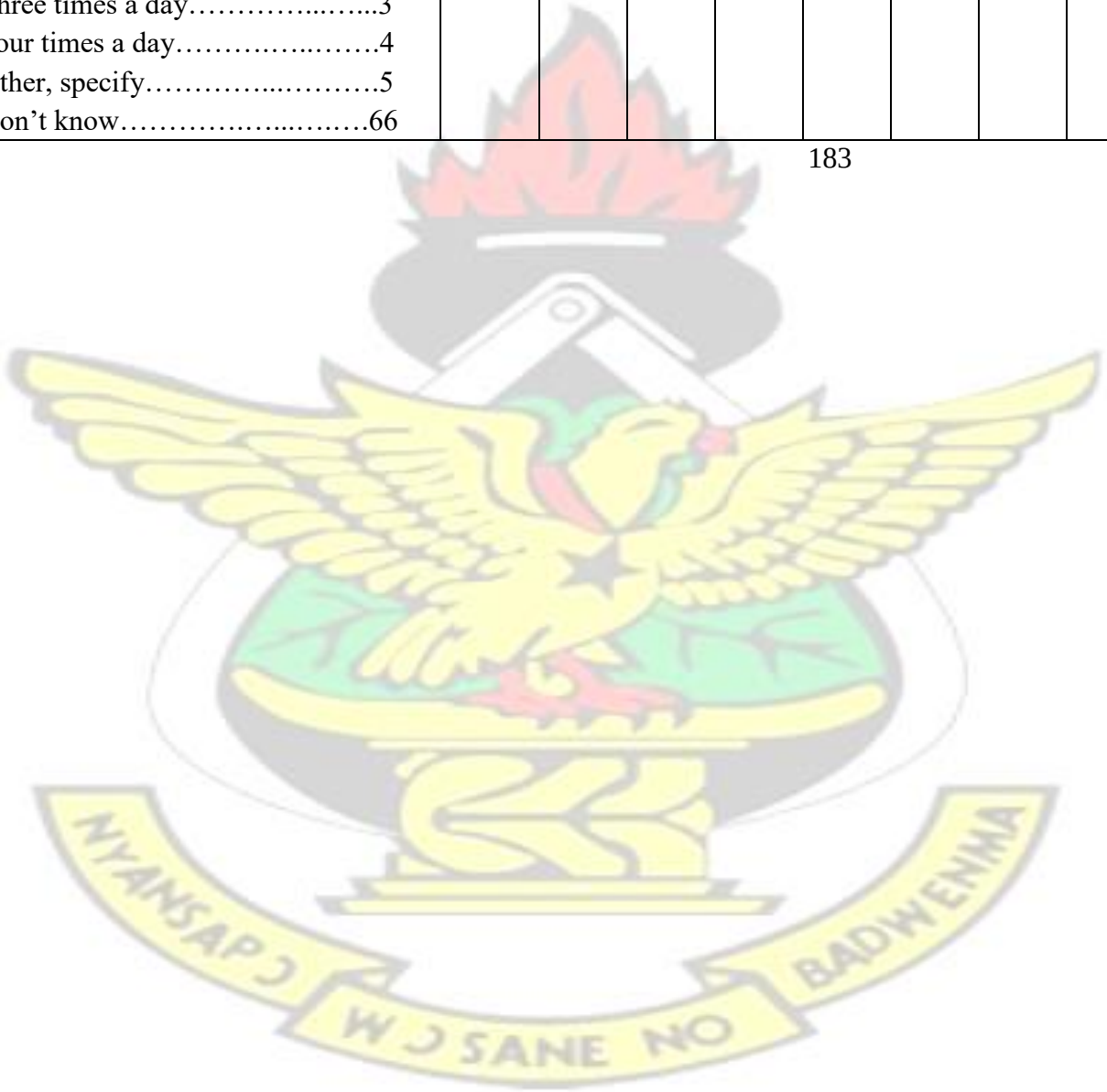
Traditional Healer…….....…10 Traditional Birth Attendant….....11 Other, Specify……..…....12												
3.10 FORM Tablet……..…1 Capsule……..……2 Syrup/Suspension……..……3 Dispersible granules……..…4 Injection……...…5 Suppository……...……6 Other, specify……...…7								F_MED				
3.11 DOSE								D_MED				

3.12 FREQUENCY Once a day…….....……1 Twice a day……...……2 Three times a day……...…...3 Four times a day…….…..……4 Other, specify……...…….5 Don't know…….…...…….66								F_MED				



question	Response	Skip	Code
3.14 Did participant do a blood test for malaria before being given medication	Yes…….....……..1 No…….....……..2		
If yes, which type of test?	RDT…….....…..1 Microscopy…….....……2 Don't know…….....……...3 Other, specify…….....……4		
3.15 How is participant now	Recovered…….....….1 Has not recovered but ok…….....….2 Sick……..…..3 Don't know……..99		
4.0 Axillary Temperature of participant 37 C? (Fever)?	Yes, specify……..……..1 No, Specify…….……2		
Is participant confirmed pregnant or delivered in the past 12 weeks	Yes…….....……...1 No…….....……2 N/A…….....…….88		
Source of confirmation	Antenatal record…….....……..1 TBA…….....……..2 Other…….....……3		
gestation	1-12 weeks, specify……..…1 13-25 weeks……...…….2 26 weeks and above……...…….3 Delivered…….4		
IPTp uptake	IPTp1…….....…….1 1PTp2…….....….2 IPTp3…….....…3 None ……..……4 Don't know……..…….66 Others, specify……5		
Source of IPTp	Health facility Antenatal clinic……..1 Maternity home……..2 TBA……..……..3 Drug Store……..4 Don't know……..…66		
Dose of IPTp	3 tablets once…….1 2 tablets once…….2 Others, specify……...3 Don't know……...…..66		

4.0 CLINICAL EXAMINATION

Examiner code……..

Weight kg	Height cm	Axillary temperature C	Abdominal examination, Spleen size cm	Liver enlargement cm

Blood sample

INSTRUCTIONS TO INTERVIEWER- explain to participant for permission to take blood sample for malaria test, explain that it is voluntary, and the results will be given to patient and used only for research purposes and will be kept confidential. Explain procedure to participant and gently take finger prick sample for Hemoglobin, blood film and RDT. Give participant his/her copy of results and explain results.

Laboratory officer code…….

Field supervisor checked	FSCODE	COMMENTS

Blood sample taken?	Blood film prepared?	Filter paper sample taken?	Hemoglobin level	RDT Results	Lab form filled?
Yes……1 No, specify.2	Yes……1 No, specify .2	Yes?.....1 No, Specify…….2		Positive….1 Negative…2 Other, specify…3	Yes no